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FACULTAD DE CIENCIAS EXACTAS Y NATURALES

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Systematics of the *Pristimantis phoxocephalus* species complex (Anura, Craugastoridae) in Ecuador

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Certifico que la disertación de Licenciatura en Ciencias Biológicas de la candidata Nadia Páez ha sido concluida de conformidad con las normas establecidas; por lo tanto, puede ser presentada para la calificación correspondiente.

Dr. Santiago R. Ron

Director de la disertación

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A mi familia

Systematics of the *Pristimantis phoxocephalus* species complex (Anura, Craugastoridae) in Ecuador

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Abstract

Pristimantis is the most diverse genus of amphibians with 473 species distributed in Central and South America. Nonetheless, this number likely underestimates the actual diversity because *Pristimantis* is expected to contain a large proportion of cryptic species. Based on integrative analysis, including molecular, morphological and bioacoustic data, this study aims to determine the species richness within the *Pristimantis phoxocephalus* species complex, a clade distributed in the southern and central Andes of Ecuador. Our results demonstrate that the *Pristimantis phoxocephalus* species complex comprises *Pristimantis phoxocephalus*, *P. atratus*, *P. ceuthospilus*, *P. versicolor*, seven confirmed candidate species and one unconfirmed candidate species; this represents an increase in species richness of 175 to 200%. Our results also demonstrate a current misapplication of the name *Pristimantis riveti*. The more accurate delimitation of the species boundaries shows that each species has a restricted distribution range. As a result, several of the redefined species could be threatened with extinction in contrast with *P. phoxocephalus sensu lato*, which is considered Least Concern. Our results provide new reasons to increase conservation efforts for these species and their habitat.

Resumen

Pristimantis es el género más diverso de anfibios del mundo con 473 especies distribuidas en América Central y América del Sur, sin embargo esta cifra subestima la diversidad real del género debido a que se especula que *Pristimantis* tiene una gran proporción de especies crípticas. Este estudio pretende determinar la riqueza de especies dentro del complejo de especies *Pristimantis phoxocephalus*, distribuido en los Andes del sur y centro del Ecuador, en base a análisis integrativos, incluyendo datos moleculares, morfológicos y bioacústicos. Nuestros resultados muestran que el complejo de especies *Pristimantis phoxocephalus* está conformado por *Pristimantis phoxocephalus*, *P. atratus*, *P. ceuthospilus*, *P. versicolor*, siete especies candidatas confirmadas y una especie candidata no confirmada. Esto que significa un incremento en la diversidad de este complejo de 175 a 200%. Los resultados también evidencian una actual aplicación errónea del nombre *Pristimantis riveti*. La determinación más precisa de los límites entre especies muestra que cada una tiene un rango de distribución más restringido. Por lo tanto varias especies podrían estar amenazadas con extinguirse, en contraste con *P. phoxocephalus sensu lato* que está en la categoría Preocupación Menor. Nuestros resultados constituyen un nuevo argumento para la aumentar los esfuerzos de conservación de estas especies y su hábitat.

Keywords: cryptic diversity, *Pristimantis atratus*, *Pristimantis versicolor*, *Pristimantis ceuthospilus*, *Pristimantis riveti*, Andes, Ecuador.

Introduction

Pristimantis (Jiménez de la Espada 1870) is the genus of amphibians with the highest species richness including 473 species worldwide (Frost 2014). It covers from Bolivia to Central America, from sea level to elevations greater than 4000 m.a.s.l. (Heinicke *et al.* 2009). In Ecuador it comprises 31% of the anuran diversity with 169 species, of which 89 are endemic to the country (Ron *et al.* 2014).

The use of DNA sequences in combination with independent sets of characters (e.g., external morphology, behavior) has allowed objective species delimitation in the framework known as integrative taxonomy (Will *et al.* 2005, Bickford *et al.* 2007, Padial *et al.* 2010). This approach has been increasingly applied to Neotropical amphibians resulting in the discovery of large numbers of cryptic species (e.g. Ron *et al.* 2006, Fouquet *et al.* 2007, Elmer and Cannatella 2009, Vieites *et al.* 2009, Funk *et al*. 2012, Caminer and Ron 2014). *Pristimantis* systematics is problematic due to the large number of cryptic species (Elmer and Cannatella 2009). Therefore the use of genetic characters on systematic reviews of *Pristimantis* is particularly promising to curate the taxonomy of the group (e.g., Elmer and Cannatella 2009, Padial and de la Riva, 2009).

A species of *Pristimantis* that is likely to contain cryptic diversity is *P. phoxocephalus*, a species reported from the Andes of Ecuador and Perú between 1800 and 3400 m.a.s.l. (Lynch 1979; Duellman and Lehr 2009). *Pristimantis phoxocephalus* was considered a member of the *Pristimantis unistrigatus* group (Lynch and Duellman 1997, Hedges *et al.* 2008) until recent studies (Padial *et al.* 2014) in which this species was removed from it and is currently unassigned to a particular species group. Recent phylogenies encompassing numerous species of *Pristimantis* (e.g. Pyron and Wiens 2011, Padial *et al.* 2014) have exposed *Pristimantis atratus*, *Pristimantis versicolor* and *Pristimantis riveti* to be relatives of *Pristimantis phoxocephalus*. We incorporated these species to the analysis and added morphologically similar specimens.

This study aims to determine the number and identity of species within the species complex *Pristimantis phoxocephalus* by integrative analysis, including genetic, morphological and bioacoustic information.

Methods

Sampling

We sampled 208 specimens from 34 localities stored at Museo de Zoología de la Pontificia Universidad Católica del Ecuador (QCAZ) (Supplementary file 1: List of individuals used for the study). Specimens examined had been previously identified as *Pristimantis phoxocephalus* (Lynch 1979), *P. atratus* (Lynch 1979), *P. ceuthospilus* (Duellman and Wild 1993), *P. riveti* (Despax 1911), *P. versicolor* (Lynch 1979) and various specimens identified as *Pristimantis* sp. The distribution of these specimens covers Andean slopes of Ecuador from Provincia Cotopaxi to Provincia Loja between 1480–3900 m.a.s.l. (Fig. 1)

Integrative analysis

In order to estimate the number of extant species in the *Pristimantis phoxocephalus* complex, we reviewed the congruence of the results of different lines of evidence under the

framework of integrative taxonomy (Dayrat 2005, Vieites *et al*. 2009). Our dataset consisted of genetic, morphological and bioacoustic characters (Cocroft and Ryan 1995). We assumed that covariation between independent sets of characters is an indication of evolutionary independence between lineages (Vieites *et al*. 2009).

To determine which clades could be considered candidate species, we obtained uncorrected pairwise *p* genetic distances for the gene 16S with Mesquite v2.75 (Maddison and Maddison 2011). We assumed that distances above 0.03 were indicative of candidate species (Fouquet *et al.* 2007).

The assignment of species status follows Vieites *et al.* (2009). Each candidate species was categorized as an unconfirmed candidate species (UCS) when there was no further information than genetic divergence; a confirmed candidate species (CCS) when the genetic variation covaried with morphological and/or bioacoustic characters; or a deep conspecific linage (DCL) when morphological and bioacoustic data did not covary with the genetic characters. We follow the species concept proposed by de Quiroz (2007) that states a species to be a separately evolving metapopulation lineage.

Phylogenetic analyses

The phylogenetic analyses were based on DNA sequences of the nuclear gene RAG1 and mitochondrial genes 16S rRNA (16S), NADH dehydrogenase subunit 1 (ND1) and 12S rRNA (12S). Fifty specimens from 40 localities were sampled, of which 22 were used as outgroups. The information of the specimens and GenBank accession numbers are given in Table 1. The alignment of the sequences was done with the plugin MAFFT (Katoh *et al.* 2002) in GeneiousPro 5.4.6 (Drummond *et al.* 2011) and a posterior manual alignment with Mesquite v2.75. The software PartitionFinder v1.1.1 (Lanfear *et al.* 2012) was used to find the best-fit partitioning schemes and their models of molecular evolution under the greedy algorithm and the Bayesian information criterion; the resulting partition scheme and chosen substitution models are summarized in Table 2.

Phylogenetic analyses were performed under two approaches, Bayesian Inference (BI) and Maximum-likelihood (ML). The Bayesian analysis was carried out with Mr. Bayes v.3.2. (Ronquist *et al.* 2012) in two simultaneous replicates for 2×10^7 generations; each replicate used four Monte Carlo Markov Chains, and the temperature parameter was set on 0.5. Trees were sampled every 1000 generations. We evaluated the convergence of the chains by plotting ln*L* and confirming it has reached stationarity; and checking the Effective Sample Size to be greater than 200 with the software Tracer v.1.5. (Rambaut and Drummond 2009). We used a 10% burn-in of the 40000 trees sampled and the remaining trees where combined to find the posterior probabilities (BPP) of a 50% majority-rule consensus tree.

For the ML analysis we used Garli v2.0 (Zwickl 2006) with ten replicates for 5×10^6 generations. We performed an intensive search using a stepwise-addition starting tree, and programmed it to finish 100000 generations after there were no topology improvements, and used default values for the rest of the parameters. Non parametric bootstraps (NPB) were evaluated with 500 bootstraps and two replicates.

Morphological characters

Only adult specimens were used in morphological analyses (Supplementary file 1). We determined the adulthood of a male by the presence of vocal sacs, vocal slits or nuptial pads; when these characters were absent we examined the gonads and categorized an individual as adult if the testes were swollen and enlarged. Females were classified as adults by the presence of large ovarian eggs; when they were not evident, we considered strongly convoluted oviducts as a feature of adulthood (Duellman and Lehr 2009).

The qualitative morphological traits included in the analysis correspond to the standardized diagnostic characters for *Pristimantis* (e.g. Duellman and Lehr 2009). Morphometric characters measured were snout–vent length (SVL), head length (HL) -from the angle of lower jaw to tip of snout-, head width (HW) and tympanum diameter (TY) (e.g. Lynch 1979, Duellman and Lehr 2009). Morphometric measurements were taken with a digital caliper with a precision of 0.01mm.

To compare clades by morphometric information, we performed pairwise Student's t-tests for each variable. For HL, HW and TY we carried out simple linear regressions between SVL and each of this variables and used the residuals for the t-test analyses, so they could be free of the effects of the body size. Statistical analyses were performed with the software JMP v.9 (Kim 1992).

Bioacoustics

Nine recordings were taken from QCAZ audio library and are available through the AmphibiaWebEcuador website (http://zoologia.puce.edu.ec/vertebrados/anfibios/). These recordings corresponds to the specimens: QCAZ47397, QCAZ47398, QCAZ47487, QCAZ25105, QCAZ45178, QCAZ46977, QCAZ46980, QCAZ46981 and one recording of an unknown specimen from the locality Cashca Totoras (recording code: cc037). We measured five variables of five randomly chosen calls (repetitive series of notes) from each recording. One of them was treated as a qualitative variable: the number of notes per call, because it depends on individual's behavior, probably influenced by the distance of the person that is recording, the proximity of conspecific or heterospecific individuals, or other uncontrolled conditions of the recordings. Quantitative variables measured were (1) duration of the note, (2) distance between notes, (3) peak time, and (4) dominant frequency (Hz). The recordings were processed with Raven Pro v.1.3 (Cornell Lab of Ornithology 2003-2008) using a sampling rate of 44.1 kHz and a frequency resolution of 10.8–11.2 Hz. Temporal data was measured on oscillograms and frequency data on power spectrums (e.g. Caminer and Ron 2014).

Results

Phylogeny and genetic variation

Tree topologies under ML and Bayesian approaches were similar (Figs. 2 and 3). Phylogenetic results reveal *Pristimantis spinosus* as sister to the *Pristimantis phoxocephalus* complex with a strong support in BI and ML approaches (1 BBP, 100% NPB). The genetic divergence analysis for the ingroup, summarized in Table 3, shows eleven clades with genetic distances above the 0.03 threshold for delimiting candidate species (Fouquet *et al.* 2007) and one clade with just 0.02 but a contrasting morphology, resulting twelve candidate species. The monophyly of each candidate species has strong support (1 BBP, 100% NPB) (Figs. 2 and 3).

Morphology

Data of qualitative morphological traits that help distinguish species within the group are shown in Table 4. All species of the *P. phoxocephalus* species complex share: tympanic membrane and prominent tympanic annulus; absence of cranial crests; finger I shorter than finger II; fingers bearing lateral fringes; ulnar tubercles low and diffuse (except for Clade I that has conical tubercles all over its body) sometimes forming an inconspicuous fold and sometimes apparently absent (all these states are present at the same time in the same clade maybe due to polymorphism of this character or to preservation effects); outer and inner edge of tarsus with low and diffused tubercles (exc. Clade G); toe V much longer than toe III; toes bearing lateral fringes. Photographs of specimens of each clade are shown in Figures 4, 5 and 6 except clades C and K for which this information is not available.

Summarized information of morphometrics is displayed in Tables 5 and 6. There is a clear morphometric differentiation between candidate species, each one being significantly different from the rest at least by one variable, according to Student's t-test analyses. These results will be mentioned when qualitative morphology is not enough to differentiate between clades. Comparative plots of morphological measurements of between clades are shown in Figures 7 and 8.

Advertisement calls

Pristimantis phoxocephalus is known for its characteristic call, a series of sharp whistles (Lynch 1979); three clades of *P. phoxocephalus* complex present this pattern, nonetheless *Pristimantis ceuthospilus*, another member of this complex, has a very different advertisement call, consisting of a sequence of several rachet-like clicks (Figs. 9 and 10). Two candidate species, Clade B and C, couldn't be differentiated by bioacustic variables because the range of note duration, peak time, distance between notes and dominant frequency overlapped. Bioacoustic data is summarized in Table 7 and plots of variable comparisons are shown in Figure 11.

Species boundaries

Based on an integrative analysis, we conclude that the *Pristimantis phoxocephalus* species complex is formed by four previously described species (*Pristimantis phoxocephalus*, *P. atratus*, *P. ceuthospilus*, *P. versicolor*), seven confirmed candidate species and one unconfirmed candidate species listed below.

Clade A: Pristimantis ceuthospilus

Clade A corresponds to *Pristimantis ceuthospilus* (Duellman and Wild 1993) and is the basal clade of *P. phoxocephalus* group with a significant support in BI (0.87 BPP). Distinctive qualitative morphological characters of this clade are the marked large pale spots -orange or yellow when alive- present on the posterior surface of the thighs and groins; and the unpigmented venter that allows seeing the abdominal muscles through the skin (in preservative) (Figs. 5 and 6). Advertisement calls of *Pristimantis ceuthospilus* are very different from other candidate species within the *P. phoxocephalus* complex. The call consists of clicks instead of the longer whistle-like notes characteristic of the other species in this study (Figs. 9 and 10). Duration of notes, peak time and distances between notes

were shorter than those of clades B, C and L; dominant frequency was the highest (Fig. 11). This is the first record of *Pristimantis ceuthospilus* from Ecuador. Specimens were found at southern Loja between 1480 and 1550 m.a.s.l. (Fig. 1).

Clade B: CCS1

Members of Clade B were previously referred as *Pristimantis phoxocephalus*. This clade occurs in Azuay province at 1800–2900 m.a.s.l., further south from the type locality of *Pristimantis phoxocephalus*: Cotopaxi province. Morphologically it is very similar to Clade C (*Pristimantis phoxocephalus*) but they differ in having dorsal skin shagreen with minute, round, low tubercles being less conspicuous in the flanks (dorsal skin in *P. phoxocephalus* is shagreen with larger and more elevated tubercles, being flatter in the flanks). The shape of the snout in lateral view is less protruding than in *P. phoxocephalus* and discs are elliptic to truncate (round in *P. phoxocephalus*). Morphometrically Clade B has a shorter SVL than *P. phoxocephalus* (Student's t test, $p = 0.02$, $t = 2.35$, $DF = 98$), has a wider (Student's t test, $p = 0.0006$, $t = -3.5$, $DF = 134$) and shorter (Student's t test, $p = 0.0015$, $t = 3.25$, $DF = 134$) head and has a larger tympanum (Student's t test, $p = 0.0001$, $t = -4.02$, $DF = 134$); Figs. 7 and 8. Advertisement calls between Clade B and *P. phoxocephalus* are similar.

Clade C: Pristimantis phoxocephalus

Clade C corresponds to *Pristimantis phoxocephalus* (Lynch 1979). Specimens included in our study have a distribution range from Cotopaxi to Chimborazo and Bolivar provinces at elevations from 2500 to 3200 m.a.s.l. *Pristimantis phoxocephalus* is similar to Clade B (see differences above) and Clade L due to the presence of a vertical keel in the snout (Fig 4). *Pristimantis phoxocephalus* differs from Clade L by its smaller size (SVL = 26.83 ± 1.82 mm. vs. 29.62 ± 2.49 mm. for males).

Clade D: Pristimantis atratus

Clade D corresponds to *Pristimantis atratus* (Lynch 1999). It occurs on the eastern slopes of the Andes in Loja and Zamora Chinchipe provinces between 2500–2812 m.a.s.l. It is easily recognizable from other species of the *P. phoxocephalus* group by having thick, conspicuous and continuous dorsolateral folds and large and conical calcars in the heels. This species has a characteristic coloration: in preservation, the dorsum is pale gray with tiny black spots –cream when alive; hidden posterior surfaces of thighs, shanks and tarsus, groin and axilla are black with large pale spots (bright yellow when alive) (Figs. 4, 5 and 6).

Clade E: CCS2

Clade E, sister group of Clade D, was found only at Tapichalaca Reserve in Zamora Chinchipe province. Clade E is easily recognizable by morphological features. Snout is rounded in lateral view and has a small papilla on its tip (Fig. 4). Its ventral coloration is cream always mottled with black (Fig. 5), dorsal coloration is brown often mottled with black (Fig. 6). Discs dilation on toes is fairly smaller than on fingers; in the other clades, discs are only slightly smaller.

Clade F: CCS3

Individuals of Clade F, along with Clades H and K, have been previously referred as *Pristimantis riveti* (Despax 1911) according to the description of this species by Lynch (1979). However, comparisons with photographs and the description of the holotype MNHNP 1902.357 make evident that none of these clades corresponds to *P. riveti*. Moreover, members of this clade inhabit Andean eastern slopes of Azuay and Morona Santiago provinces at 3342–3670 m.a.s.l. while the holotype of *Pristimantis riveti* was sampled further north in Tungurahua province. Clade F is morphologically similar to Clade H and K. They differ from the rest of species in the *P. phoxocephalus* complex by their brown coloration with scattered large black spots and having the smallest discs on fingers and toes in the group (Fig. 6). Comparisons between Clade F, H and K are discussed on the Clade H account.

Clade G: CCS4

Clade G occurs in El Oro province at 3163 m.a.s.l. It is sister to Clade F, with strong support (1 BPP; 100% NPB) (Fig. 3) and their pairwise genetic distances is 0.02. Nevertheless, Clade G was considered a confirmed candidate species because its morphology is contrastingly different from its sister species and the rest of the clades. Clade G differs from F by being significantly larger (Student's t test, $p = 0.005$, $t = 2.98$, $DF = 29$), having wider digital pads and having a reddish dorsal coloration when alive (Fig. 6). Morphometrically Clade G has a smaller tympanum than F and the rest of the clades (Fig. 8).

Clade H: CCS5

Clade H inhabits the western slopes of the Andes of Azuay province at 3760 m.a.s.l. It is distinguished from Clade F by the texture of its dorsal skin, being smooth and forming low ridges in Clade H, but shagreen with small, rounded, medium elevated tubercles in F. Spots on the posterior surfaces of the thigh are conspicuous in clade H while in F they are generally smaller and less evident; discs of fingers and toes are slightly narrower than those in Clade F; body and limbs of Clade H are sturdier than those in F (Fig. 6). Males of Clade F are significantly larger than Clade H (Student's t test, $p = 0.0002$, $t = 3.85$, $DF = 98$). When compared with Clade K there were no significant differences in morphology. Therefore, we consider Clade H as a confirmed candidate species and Clade K as an unconfirmed candidate species. Genetic distances between both clades are > 5% (Table 3).

Clade I: CCS6

Clade I represents the easternmost record of the *P. phoxocephalus* complex. It occurs in Napo province at an elevation of 2880 m.a.s.l. Clade I does not resemble any other species from *Pristimantis phoxocephalus* complex. It has medium sized, conical, high tubercles on the dorsum, limbs and eyelids. Dorsal coloration when alive is green with black chevrons (Fig. 6).

Clade J: Pristimantis versicolor

Clade J corresponds to *Pristimantis versicolor* described by Lynch (1979). Specimens are distributed in Zamora Chinchipe and Loja provinces at an altitude of 2100–3200 m.a.s.l. *Pristimantis versicolor* is easily distinguished from other members of the *Pristimantis*

phoxocephalus complex because it is the only species of the group whose adult males lack a vocal sac and vocal slits. In addition, they have a median, subconical, prominent tubercle in the heel. Morphometrically this clade has the smallest snout-vent length of the group (Fig. 7).

Clade K: UCS1

Since Clade K is represented just by a single specimen, we do not have enough information about its morphological variation; therefore Clade K could not be morphologically differentiated from Clade H despite their high genetic distances of 0.55 (Table 3). This coupled with the fact that Clade K and H coexist in the same habitat, leads us to consider Clade K as an unconfirmed candidate species, until new ecological, behavioral or morphological information is available. Clade H was considered the confirmed candidate species because it was the clade with more available information.

Clade L: CCS7

Clade L is distributed in the Andean western slopes of Azuay between 2900 and 3660 m.a.s.l. This is by far the largest species of the *P. phoxocephalus* complex (SVL = 29.62 ± 2.49 mm. for males and 43.97 ± 5.00 mm. for females. It also has the shortest head relative to SVL (Table 6, Fig. 8). Clade L is unique for its skin texture on the flanks, which has conspicuous pustules, arranged in rows. Advertisement calls differ from the ones of Clade A, B and C; notes have the longest duration, the major peak time and the lowest dominant frequency (Fig. 11).

Discussion

Taxonomy and phylogenetic relationships

Our results indicate that previously proposed, morphology-based, relationships for species in this group are not in agreement with the phylogeny. *Pristimantis phoxocephalus* was thought to be closely related to *Pristimantis eugeniae*, *Pristimantis nyctophylax* and *Pristimantis subsigillatus* (Lynch and Duellman 1997) while *P. versicolor* was considered close to *P. cajamarcensis* and *P. unistrigatus* (Lynch 1979). *Pristimantis bellator, P. cajamarcensis, P. lirellus, P. muscosus, P. rufioculis,* and *P. stictogaster* were considered to be close to *Pristimantis ceuthospilus*. The phylogenetic tree obtained in this study shows *P. phoxocephalus, P. atratus, P. ceuthospilus* and *P. versicolor* closely related to each other. These results are partly inconsistent with recent phylogenies by Pyron and Wiens (2011), and Padial *et al.* (2014), in which *Pristimantis ceuthospilus* is not part of the *P. phoxocephalus* complex and *Pristimantis riveti* is still misidentified and considered a close relative of *Pristimantis phoxocephalus.*

Another striking result was the detection of a misapplication of the binomial *Pristimantis riveti*. CCS3, CCS5 and UCC1 were previously identified as *Pristimantis riveti* (Lynch 1979). The description relied on specimens from Azuay province (southern Ecuador), while the holotype was collected in Tungurahua province (central Ecuador). By checking measurements and photographs of the holotype: MNHNP 1902.357, and comparing them with specimens of this study, we conclude that they are different species.

Cryptic diversity

This study proves that the *Pristimantis phoxocephalus* complex comprises four previously described species, seven confirmed candidate species and one unconfirmed candidate species. This represents an increasing in species richness in the complex by 175-200%.

The underestimation of species richness in this complex results from the presence of morphologically cryptic species and the lack of taxonomic studies, since four of the new species are morphologically distinctive from previously described *Pristimantis*. Moreover, inside the QCAZ collection there are specimens from at least four populations that are morphologically similar to *Pristimantis phoxocephalus* and that may represent additional species for the complex. We could not include them in our study because they lack tissues for DNA analyses. Similarly, populations from northern Peru ascribed to *P. phoxocephalus* likely represent an additional undescribed species (Duellman and Lehr 2009).

The *Pristimantis phoxocephalus* complex has a high interspecific genetic divergence with an average uncorrected genetic distance of 0.052 for the gene 16S. Despite this, CCS3 and CCS4 exhibit a genetic distance of only 0.020, value that is under the threshold for delimiting candidate species proposed by Fouquet *et al.* (2007). The clear morphological difference of these species coupled with the low intraspecific genetic distance of each one (average of 0.002), suggest that in some cases the threshold value is too conservative to diagnose candidate species; therefore running the risk of underestimating the number of species. Other studies on cryptic diversity on Neotropical amphibians have come to the same conclusion (e.g. Coloma *et al*. 2012, Funk *et al.* 2012).

Impact on conservation

The discovery of new cryptic species represents an important argument for conservation (Bickford *et al.* 2007). One of the parameters to determine the conservation status of a species is the size of the distribution range (IUCN 2014). After dividing what was thought to be a single species into several cryptic species, the distribution of each one is going to be more restricted. Therefore the probability of extinction of each species should be higher than previous estimates. For example, *Pristimantis phoxocephalus* is catalogued as a Least Concern species by the IUCN Red List (Rodríguez *et al.* 2004). Currently it is considered to be present along the Pacific western slopes of the Andes in Ecuador and Peru at elevations of 1800-3100 m.a.s.l. (Rodríguez *et al.* 2004). Our study shows that it is restricted to the western slopes of the Andes in central Ecuador in Cotopaxi, Chimborazo and Bolivar provinces at 2500–3200 m.a.s.l. Therefore, *Pristimantis phoxocephalus* should be a species with a higher conservation concern.

The range of distribution reported for *Pristimantis riveti* by the IUCN Red List includes Andean cordilleras surrounding the Cuenca basin, from 2620-3600 m.a.s.l. and Parque Nacional Podocarpus (Coloma *et al.* 2004). This distribution is based on individuals previously identified as *Pristimantis riveti* according to the species account by Lynch (1979). In accordance with this information it is catalogued as a Near Threatened species (Coloma *et al.* 2004) but now that we know these specimens do not belong to *Pristimantis riveti* species, the conservation status should be Data Deficient since there are no records from the type locality or other localities since its first description by Despax (1911).

Before this study, *Pristimantis ceuthospilus* was reported only from Peru (Rodríguez *et al.* 2014); the new record for Ecuador expands its distribution range, so the status Least

Concern shouldn't suffer changes. The range of distribution of *Pristimantis atratus* and *Pristimantis versicolor* remains unchanged.

Accurate taxonomic assessments contribute to conservation by helping to delimit priority conservation areas. Species in our study are distributed along the central and southern Andes at high elevations in areas currently threatened by agriculture, cattle rising, dam constructions, mining, logging and human settlement (Coloma *et al*. 2004, Rodríguez *et al.* 2004). The eight new records of *Pristimantis* for the southern Andes provide new reasons for the conservation of this region.

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References

Bickford D, Lohman DJ, Sodhi NS, Ng PK, Meier R, Winker K, Ingram KK, Das I (2007) Cryptic species as a window on diversity and conservation. Trends in Ecology & Evolution 22: 148–155.

Caminer MA, Ron SR (2014) Systematics of treefrogs of the *Hypsiboas calcaratus* and *Hypsiboas fasciatus* species complex (Anura, Hylidae) with the description of four new species. ZooKeys 370: 1–68. doi: 10.3897/zookeys.370.6291.

Cocroft RB, Ryan MJ (1995) Patterns of advertisement call evolution in toads and chorus frogs. Animal Behavior 49: 283–303.

Coloma LA, Ron SR, Yánez-Muñoz M, Almeida D (2004) *Pristimantis riveti*. The IUCN Red List of Threatened Species. Version 2014.2. <www.iucnredlist.org>. Accessed on September 17, 2014.

Coloma LA, Carvajal-Endara S, Dueñas JF, Paredes-Recalde A, Morales-Mite M, Almeida-Reinoso D, Tapia EE, Hutter CR, Toral E, Guayasamín JM (2012) Molecular phylogenetics of stream frogs of the *Hyloscirtus larinopygion* group (Anura: Hylidae), and description of two new species from Ecuador. Zootaxa 3364: 1–78

Cornell Lab of Ornithology (2003–2008) Raven pro. Versión 1.3. Bioacustic Research Program. New York, United States of America.

Dayrat B (2005) Towards integrative taxonomy. Biological Journal of the Linnean Society 85: 407–415.

De Queiroz K (2007) Species concepts and species delimitation. Systematic biology 56: 879–886.

Despax R (1911) Mission géodésique de l'Équateur. Collections recueilles par M. le Dr. Rivet. Batraciens anoures. Bulletin du Museum National d'Histoire Naturelle. Paris 17: 90–94.

Drummond AJ, Ashton B, Buxton S, Cheung M, Cooper A, Duran C, Field M, Heled J, Kearse M, Markowitz S (2011) Geneious, Version 5.4; Biomatters Ltd. Auckland, New Zealand. <http://www. geneious. com>. Accessed on September 17, 2012.

Duellman WE, Lehr E (2009) Terrestrial breeding frogs (Strabomantidae) in Peru. Natur- und Tier-Verlag, Naturwissenschaft, Münster.

Duellman WE, Wild ER (1993) Anuran amphibians from the Cordillera de Huancabamba, northern Peru: Systematics, ecology, and biogeography. Occasional Papers of the Museum of Natural History, University of Kansas 157: 1–53.

Elmer KR, Cannatella DC (2009) Three new species of leaflitter frogs from the upper Amazon forests: cryptic diversity within *Pristimantis "ockendeni"* (Anura: Strabomantidae) in Ecuador. Bibliothek der Universität Konstanz.

Fouquet A, Gilles A, Vences M, Marty C, Blanc M, Gemmell NJ (2007) Underestimation of species richness in neotropical frogs revealed by mtDNA analyses. PLoS ONE 10: e1109. doi: 10.1371/journal.pone.0001109.

Frost DR (2014) Amphibian Species of the World: an Online Reference. Version 6.0. American Museum of Natural History, New York, USA. <http://research.amnh.org/herpetology/amphibia/index.html>. Accessed on September 18, 2014.

Funk WC, Caminer M, Ron SR (2012) High levels of cryptic species diversity uncovered in Amazonian frogs. Proceedings of the Royal Society, Biological Sciences 279: 1806–1814.

Hedges SB, Duellman WE, Heinicke MP (2008) New World direct-developing frogs (Anura: Terrarana): molecular phylogeny, classification, biogeography, and conservation. Zootaxa 1737: 1–182.

Heinicke MP, Duellman WE, Trueb LI, Means DB, MacCulloch RD, Hedges SB (2009) A new frog family (Anura: Terrarana) from South America and an expanded direct-developing clade revealed by molecular phylogeny. Zootaxa 2211: 1–35.

IUCN Standards and Petitions Subcommittee (2014) Guidelines for Using the IUCN Red List Categories and Criteria. Version 11. Prepared by the Standards and Petitions Subcommittee. <http://www.iucnredlist.org/documents/RedListGuidelines.pdf.>. Accessed on September 17, 2014.

Jiménez de la Espada M (1870) Fauna neotropicalis species quaedam nondum cognitae. Jornal de Sciências, Mathemáticas, Physicas e Naturae. Lisboa 3: 57–65.

Katoh K, Misawa K, Kuma KI, Miyata T (2002) MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. Nucleic acids research 30: 3059-3066.

Kim K (1992) JMP, Version 2. Software for Statistical Visualization on the Apple Macintosh. Journal of Chemical Information and Computer Sciences 32: 174–175.

Lanfear R, Calcott B, Ho SY, Guindon S (2012) PartitionFinder: combined selection of partitioning schemes and substitution models for phylogenetic analyses. Molecular biology and evolution 29: 1695–1701.

Lynch JD (1979) Leptodactylid frogs of the genus *Eleutherodactylus* from the Andes of southern Ecuador. Museum of Natural History, University of Kansas, Miscellaneous Publication 66: 1–62.

Lynch JD, Duellman WE (1997) Frogs of the genus *Eleutherodactylus* in western Ecuador. Systematics, ecology, and biogeography. Natural History Museum, University of Kansas, Special Publication 23: 1–236.

Maddison WP, Maddison DR (2011) Mesquite: A modular system for evolutionary analysis. Version 2.74 < http://mesquiteproject.org>

Padial JM, De la Riva I (2009) Integrative taxonomy reveals cryptic Amazonian species of *Pristimantis* (Anura: Strabomantidae). Zoological Journal of the Linnean Society 155: 97-122.

Padial JM, Grant T, Frost DR (2014) Molecular systematics of terraranas (Anura: Brachycephaloidea) with an assessment of the effects of alignment and optimality criteria. Zootaxa 3825: 1–132.

Padial JM, Miralles A, De la Riva I, Vences M (2010) Review: The integrative future of taxonomy. Frontiers in Zoology 7: 1–14.

Pyron AR, Wiens JJ (2011) A large-scale phylogeny of Amphibia including over 2800 species, and a revised classification of extant frogs, salamanders, and caecilians. Molecular Phylogenetics and Evolution 61: 543–583.

Rambaut A, Drummond AJ (2009) Tracer. Version 1.5. <http://beast.bio.edu.ac.uk/Tracer/.>.

Rodríguez L, Martinez JL, Ron SR, Coloma LA, Almeida D (2004) *Pristimantis phoxocephalus*. The IUCN Red List of Threatened Species. Version 2014.2. <www.iucnredlist.org>. Accessed on 17 September 2014.

Rodríguez L, Martinez JL, Wild E (2004) *Pristimantis ceuthospilus*. The IUCN Red List of Threatened Species. Version 2014.3. <www.iucnredlist.org>. Accessed on 15 September, 2014.

Ron SR, Santos JC, Cannatella DC (2006) Phylogeny of the túngara frog genus *Engystomops* (= *Physalaemus pustulosus* species group; Anura: Leptodactylidae). Molecular Phylogenetics and Evolution 39: 392–403. doi:10.1016/j.ympev.2005.11.022.

Ron SR, Guayasamin JM, Yanez-Muñoz MH, Merino-Viteri A, Ortiz DA (2014) AmphibiaWebEcuador. Version 2014.0. Museo de Zoología, Pontificia Universidad Católica del Ecuador. <http://zoologia.puce.edu.ec/Vertebrados/anfibios/Anfibios Ecuador>. Accesed on September 17, 2014.

Ronquist F, Teslenko M., van der Mark P, Ayres DL, Darling A, Höhna S, Huelsenbeck JP (2012) MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. Systematic biology 61: 539-542.

Vieites DR, Wollenberg KC, Andreone F, Köhler J, Glaw F, Vences M (2009) Vast underestimation of Madagascar's biodiversity evidenced by an integrative amphibian inventory. Proceedings of the National Academy of Sciences of the United States of America 106: 8267–8272.

Will KW, Mishler BD, Wheeler QD (2005) The perils of DNA barcoding and the need for integrative taxonomy. Systematic biology 54: 844-851.

Zwickl D (2006) Genetic algorithm approaches for the phylogenetic analysis of large biological sequence datasets under the maximum likelihood criterion. Ph.D. thesis: The University of Texas at Austin.

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Figure 1. Distribution map of the *Pristimantis phoxocephalus* complex based on specimens deposited at QCAZ. Numbers refer to localities specified in Supplementary file 1.

Figure 2. Phylogenetic tree of *Pristimantis phoxocephalus* complex showing outgroups. The ingroup is marked with a green band. Posterior probabilities followed by bootstraps support values (percentage) are shown under each branch. Values under 0.8 for posterior probabilities and 60% for bootstraps are not shown. * Indicates a support of 1 for posterior probabilities and 100% for bootstraps.

Figure 3. Phylogenetic tree of the *Pristimantis phoxocephalus* complex. Each clade is marked with a band of different color. Posterior probabilities followed by bootstraps support values (percentage) are shown under each branch. Values under 0.8 for posterior probabilities and 60% for bootstraps are not shown. Z corresponds to the closest outgroup, other outgroups are not shown. * Indicates a support of 1 for posterior probabilities and 100% for bootstraps.

Figure 4. Lateral view of adult individuals of the *P. phoxocephalus* species complex. **A** *Pristimantis ceuthospilus* (QCAZ47487, ♂, SVL: 25.4 mm.) **B** CCS1 (QCAZ45189, ♂, 25.69 mm.) **C** *Pristimantis atratus* (QCAZ45645, ♂, 27.77 mm.) **D** CCS2 (QCAZ47731, ♂, 23.13 mm.) **E** CCS3 (QCAZ56536, ♀, 31.29 mm.) **F** CCS4 (QCAZ45129, ♀, 38.32

mm.) **G** CCS5 (QCAZ42993, ♀, 30.04 mm.) **H** CCS6 (QCAZ46221, ♀, 29.48 mm.) **I** *Pristimantis versicolor* (QCAZ45650, ♀, 25.26 mm.) **J** CCS7 (QCAZ46993, ♀, 47.50 mm.).

Figure 5. Ventral view of live individuals of the *P. phoxocephalus* species complex. **A** *Pristimantis ceuthospilus* (QCAZ47487, ♂, SVL: 25.4 mm.) **B** CCS1 (QCAZ45188, ♂, 24.35 mm.) **C** *Pristimantis atratus* (QCAZ45645, ♂, 27.77 mm.) **D** CCS2 (QCAZ45669, ♂, 23.13 mm.) **E** CCS3 (QCAZ56536, ♀, 31.29 mm.) **F** CCS4 (QCAZ45129, ♀, 38.32 mm.) **G** CCS5 (QCAZ42993, ♀, 30.04 mm.) **H** CCS6 (QCAZ46221, ♀, 29.48 mm.) **I** *Pristimantis versicolor* (QCAZ45650, ♀, 25.26 mm.) **J** CCS7 (QCAZ45174, ♀, 40.43 mm.).

Figure 6. Dorsal view of live individuals of the *P. phoxocephalus* species complex. **A** *Pristimantis ceuthospilus* (QCAZ47487, ♂, SVL: 25.4 mm.) **B** CCS1 (QCAZ45170, ♂, 24.11 mm.) **C** *Pristimantis atratus* (QCAZ45645, ♂, 27.77 mm.) **D** CCS2 (QCAZ45669, ♂, 23.13 mm.) **E** CCS3 (QCAZ56536, ♀, 31.29 mm.) **F** CCS4 (QCAZ45129, ♀, 38.32 mm.) **G** CCS5 (QCAZ42993, ♀, 30.04 mm.) **H** CCS6 (QCAZ46221, ♀, 29.48 mm.) **I** *Pristimantis versicolor* (QCAZ45650, ♀, 25.26 mm.) **J** CCS7 (QCAZ46993, ♀, 47.50 mm.).

Figure 7. Comparisons of morphometric variables for each clade. **A** male SVL **B** female SVL. Mean is shown with a green line, boxplots are shown in red.

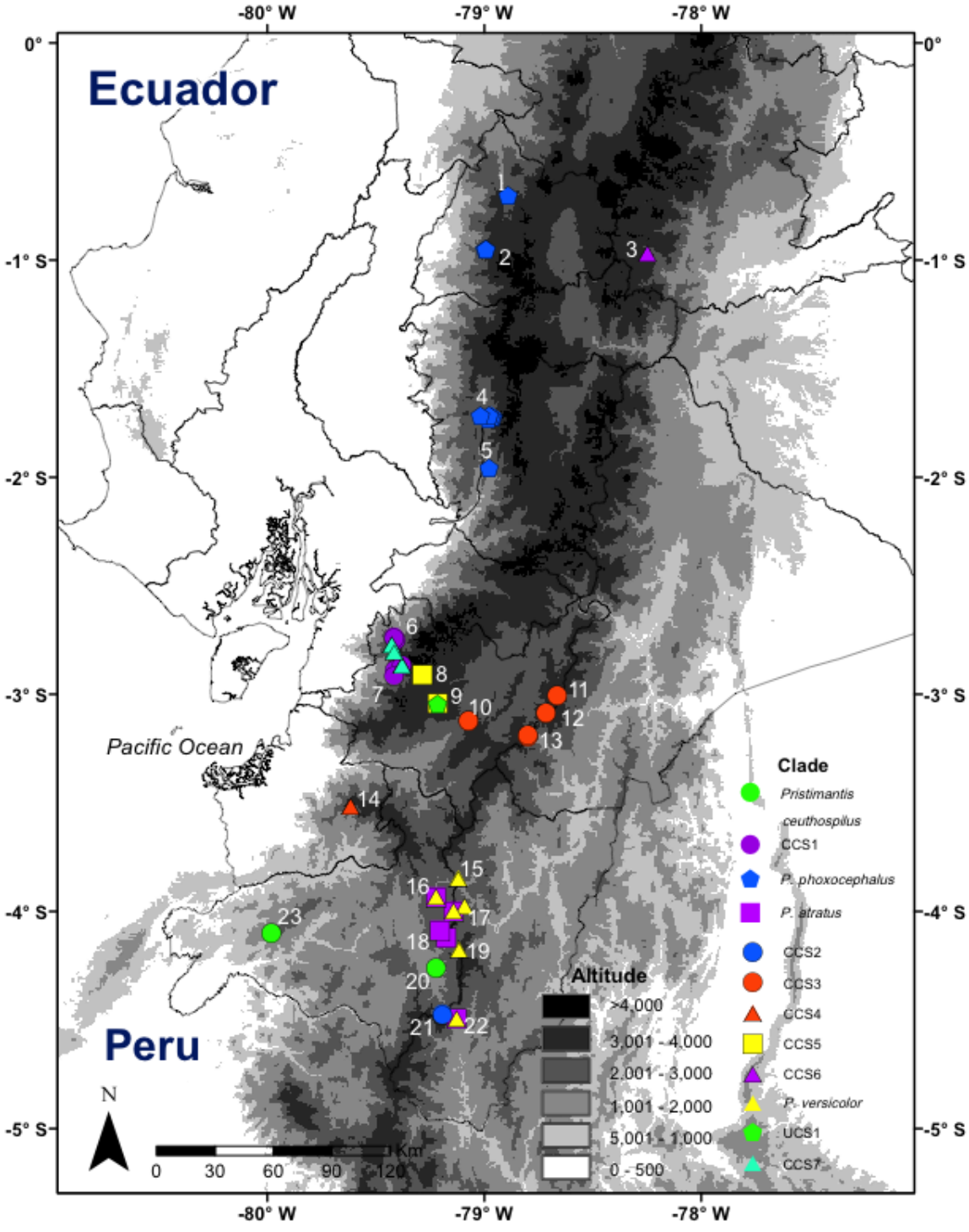
Figure 8. Comparisons of morphometric variables for each clade. **A** head length (HL) residuals **B** head width (HW) residuals **C** tympanum diameter (TY) residuals. Mean is marked with a green line, and boxplots are showed in red.

Figure 9. Oscillograms of consecutive advertisement calls of **A** *Pristimantis ceuthospilus* (QCAZ 47397) **B** CCS2 (QCAZ46977) **C** *Pristimantis phoxocephalus* (Recording from Cashca Totoras) **D** CCS7 (QCAZ46981).

Figure 10. Oscillograms (left) and spectrograms (right) of advertisement calls of **A** *Pristimantis ceuthospilus* (QCAZ 47397) **B** CCS2 (QCAZ46977) **C** *Pristimantis phoxocephalus* (Recording from Cashca Totoras) **D** CCS7 (QCAZ46981).

Figure 11. Bivariate-fit for different advertisement calls variables for clades A, B, C and L. **A** peak time (s) vs. note duration (s) **B** dominant frequency (Hz) vs. note duration (s)

Figure 1.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-78° W
0°
-1° S
-2° S
-3° S
-4° S
-5° S
Ecuador
Peru
Pacific Ocean
1
2
3
4
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N
0
30
60
90
120
Km
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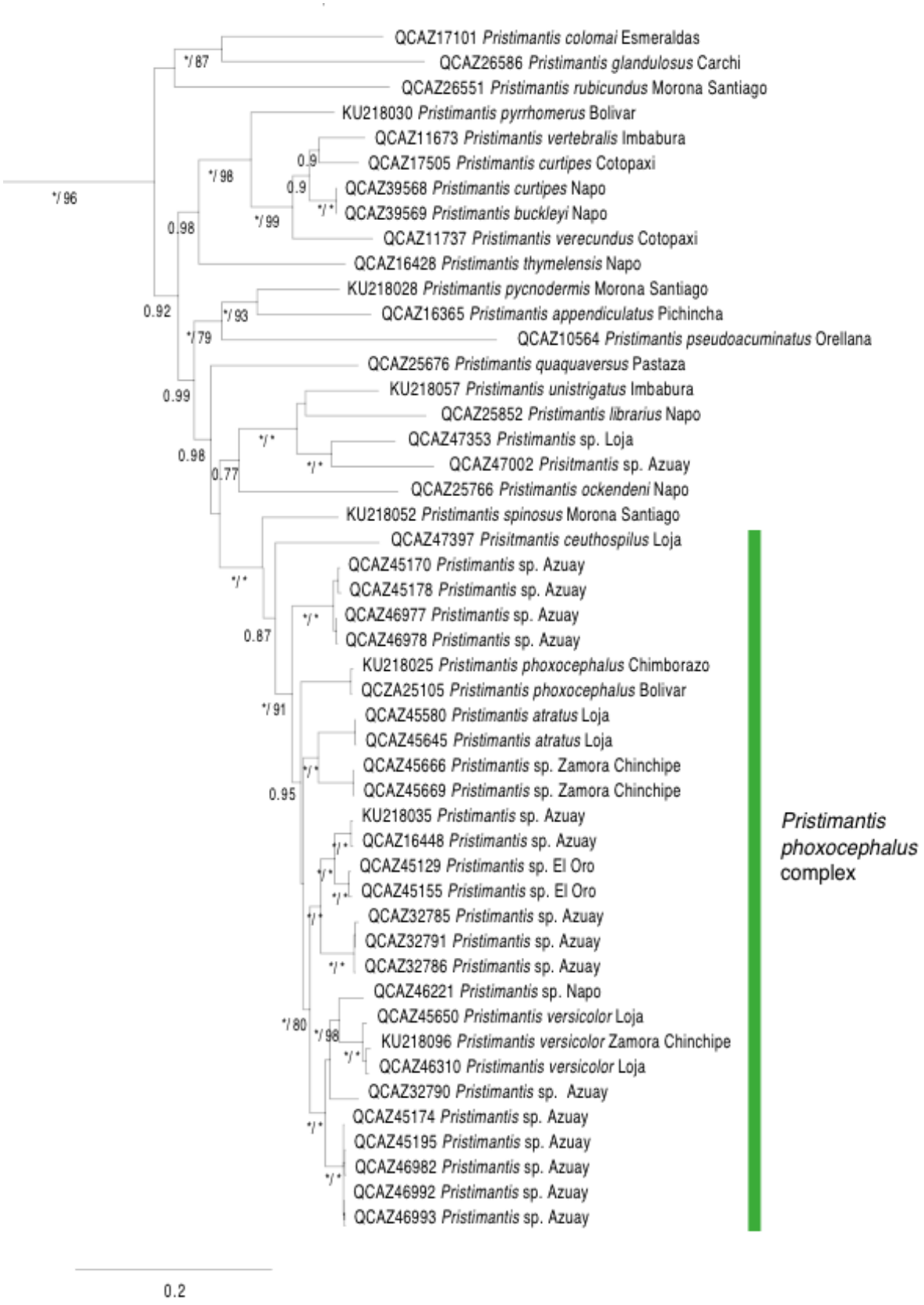
>4,000
3,001 - 4,000
2,001 - 3,000
1,001 - 2,000
501 - 1,000
0 - 500
Clade
Pristimantis ceuthospilus
CCS1
P. phoxocephalus
P. atratus
CCS2
CCS3
CCS4
CCS5
CCS6
P. versicolor
UCS1
CCS7

Figure 2.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QCAZ11673 Pristimantis vertebralis Imbabura
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QCAZ39568 Pristimantis curtipes Napo
QCAZ39569 Pristimantis buckleyi Napo
QCAZ11737 Pristimantis verecundus Cotopaxi
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KU218057 Pristimantis unistrigatus Imbabura
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QCAZ47002 Prisitmantis sp. Azuay
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KU218052 Pristimantis spinosus Morona Santiago
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QCAZ45645 Pristimantis atratus Loja
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QCAZ32791 Pristimantis sp. Azuay
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QCAZ46221 Pristimantis sp. Napo
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KU218096 Pristimantis versicolor Zamora Chinchipe
QCAZ46310 Pristimantis versicolor Loja
QCAZ32790 Pristimantis sp. Azuay
QCAZ45174 Pristimantis sp. Azuay
QCAZ45195 Pristimantis sp. Azuay
QCAZ46982 Pristimantis sp. Azuay
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QCAZ46993 Pristimantis sp. Azuay
Pristimantis phoxocephalus complex
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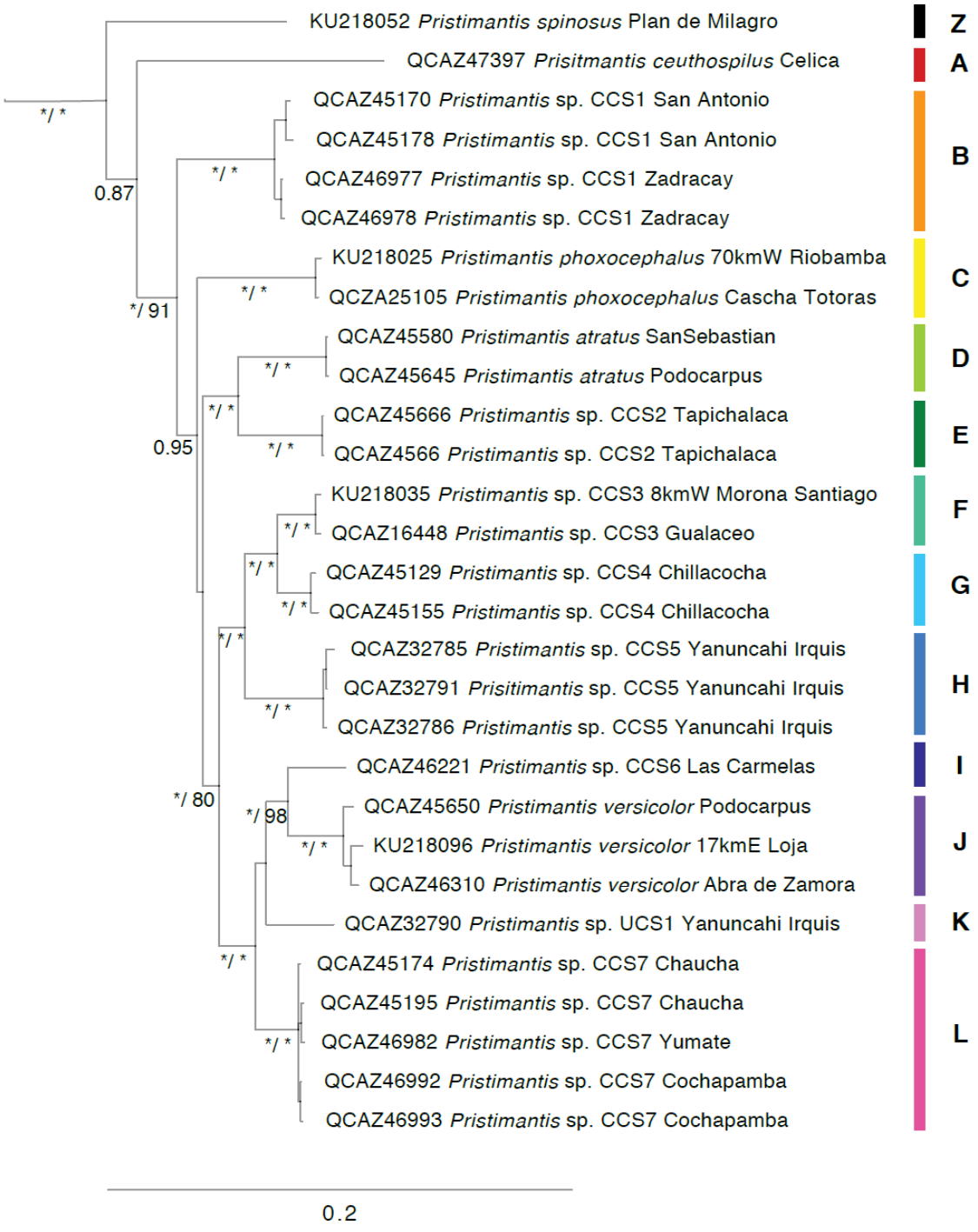
Figure 3.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KU218025 Pristimantis phoxocephalus 70kmW Riobamba
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QCAZ4566 Pristimantis sp. CCS2 Tapichalaca
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QCAZ32791 Prisitimantis sp. CCS5 Yanuncahi Irquis
QCAZ32786 Pristimantis sp. CCS5 Yanuncahi Irquis
QCAZ46221 Pristimantis sp. CCS6 Las Carmelas
QCAZ45650 Pristimantis versicolor Podocarpus
KU218096 Pristimantis versicolor 17kmE Loja
QCAZ46310 Pristimantis versicolor Abra de Zamora
QCAZ32790 Pristimantis sp. UCS1 Yanuncahi Irquis
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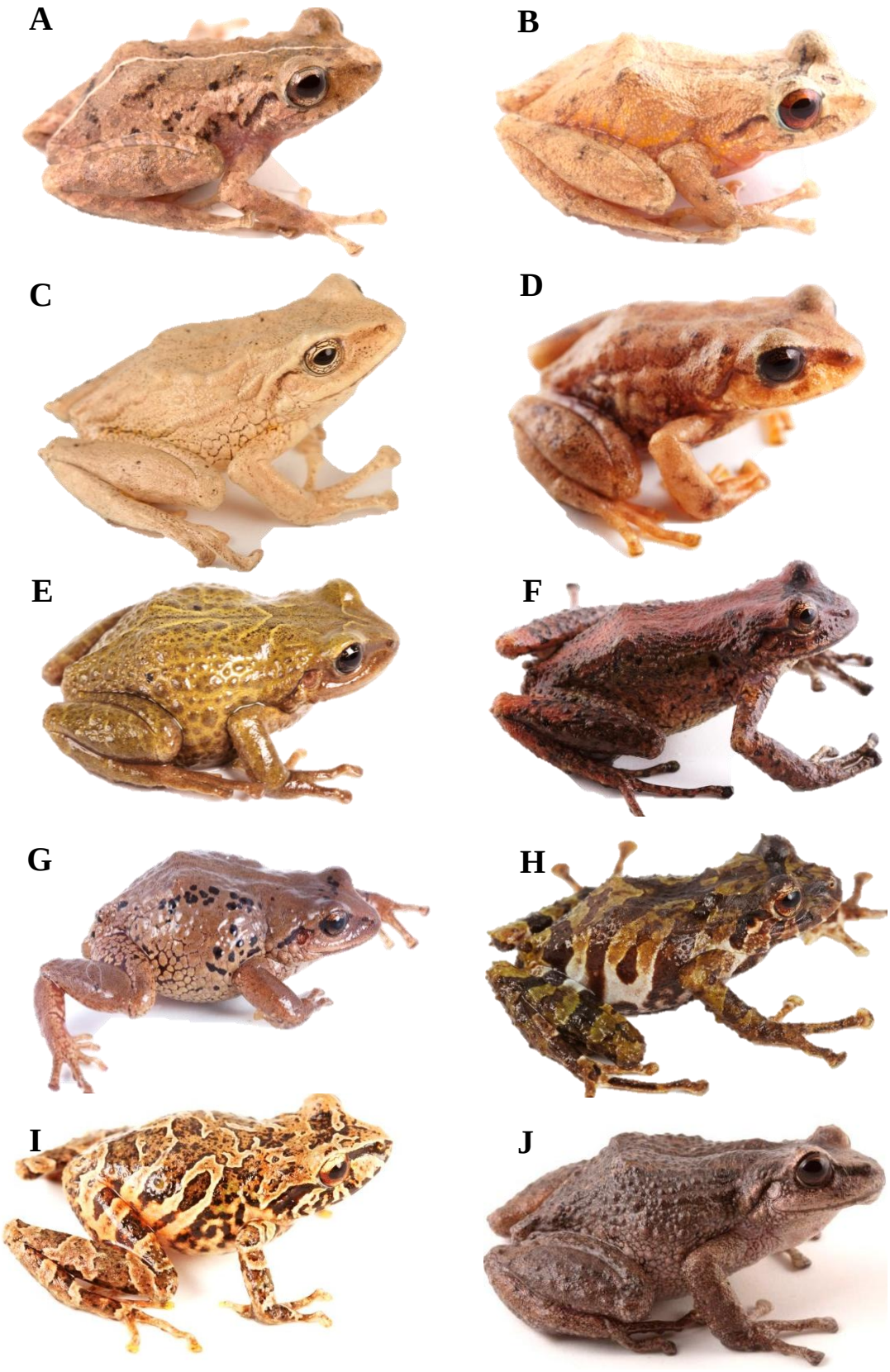
Figure 4.



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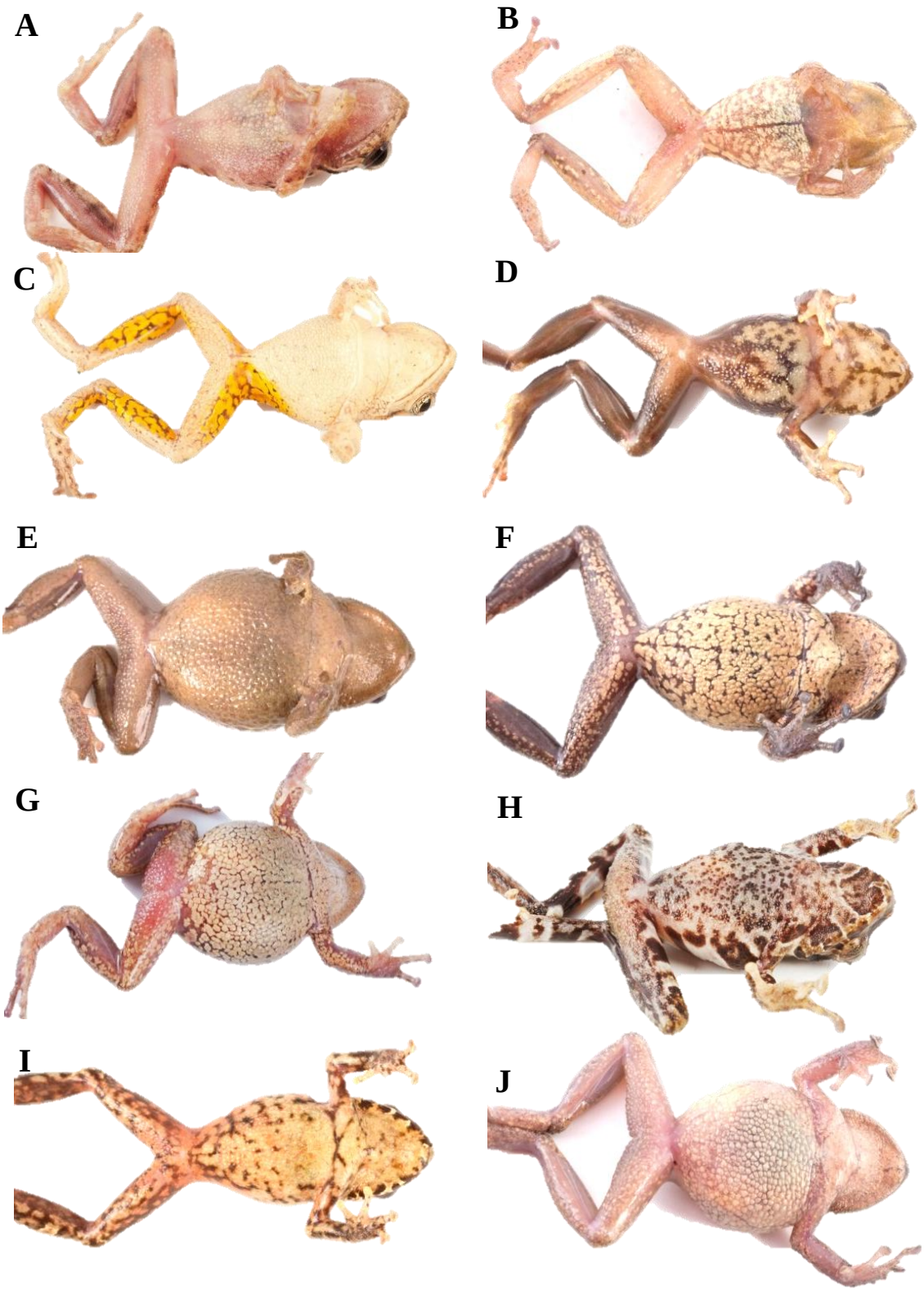

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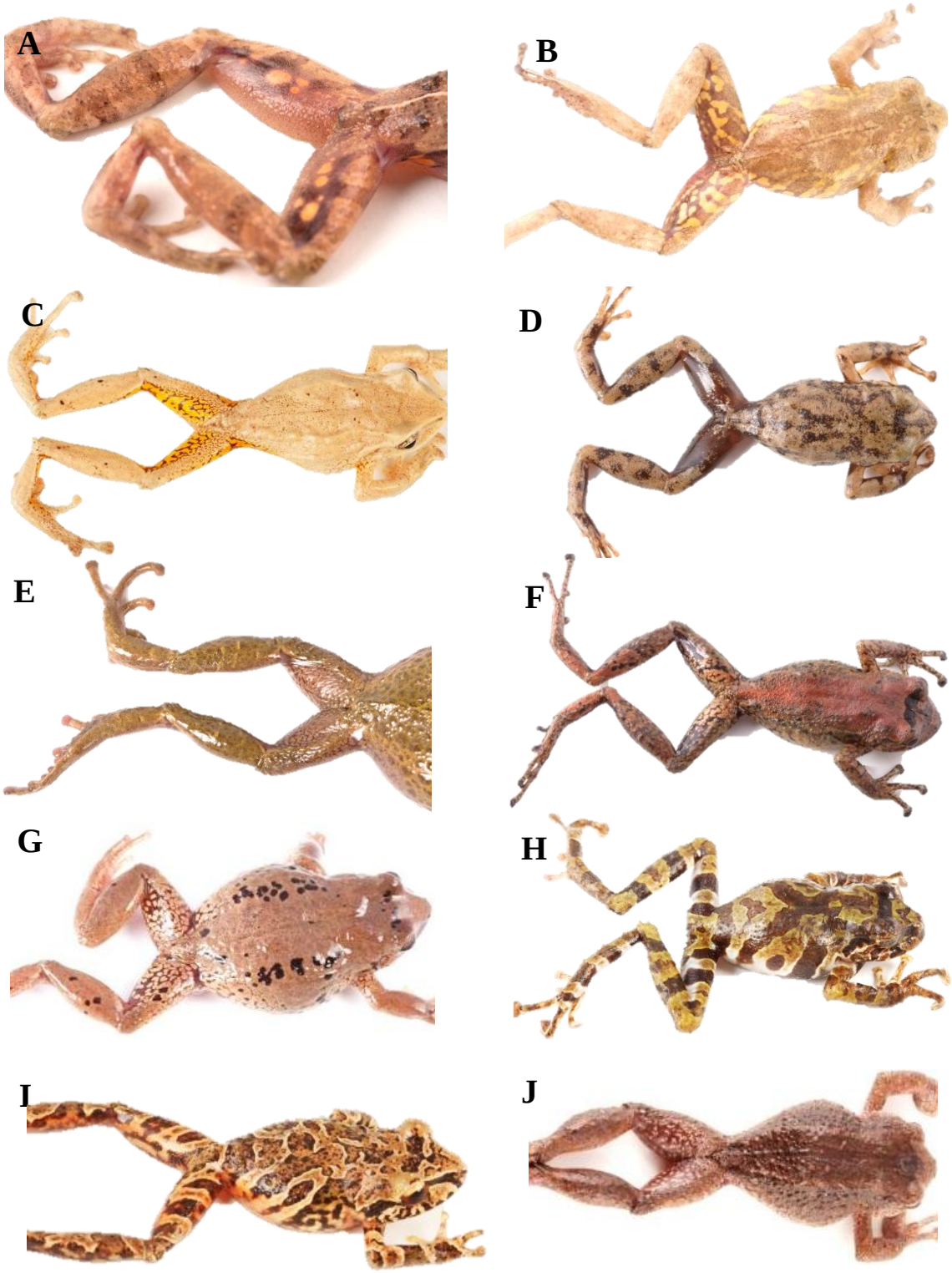

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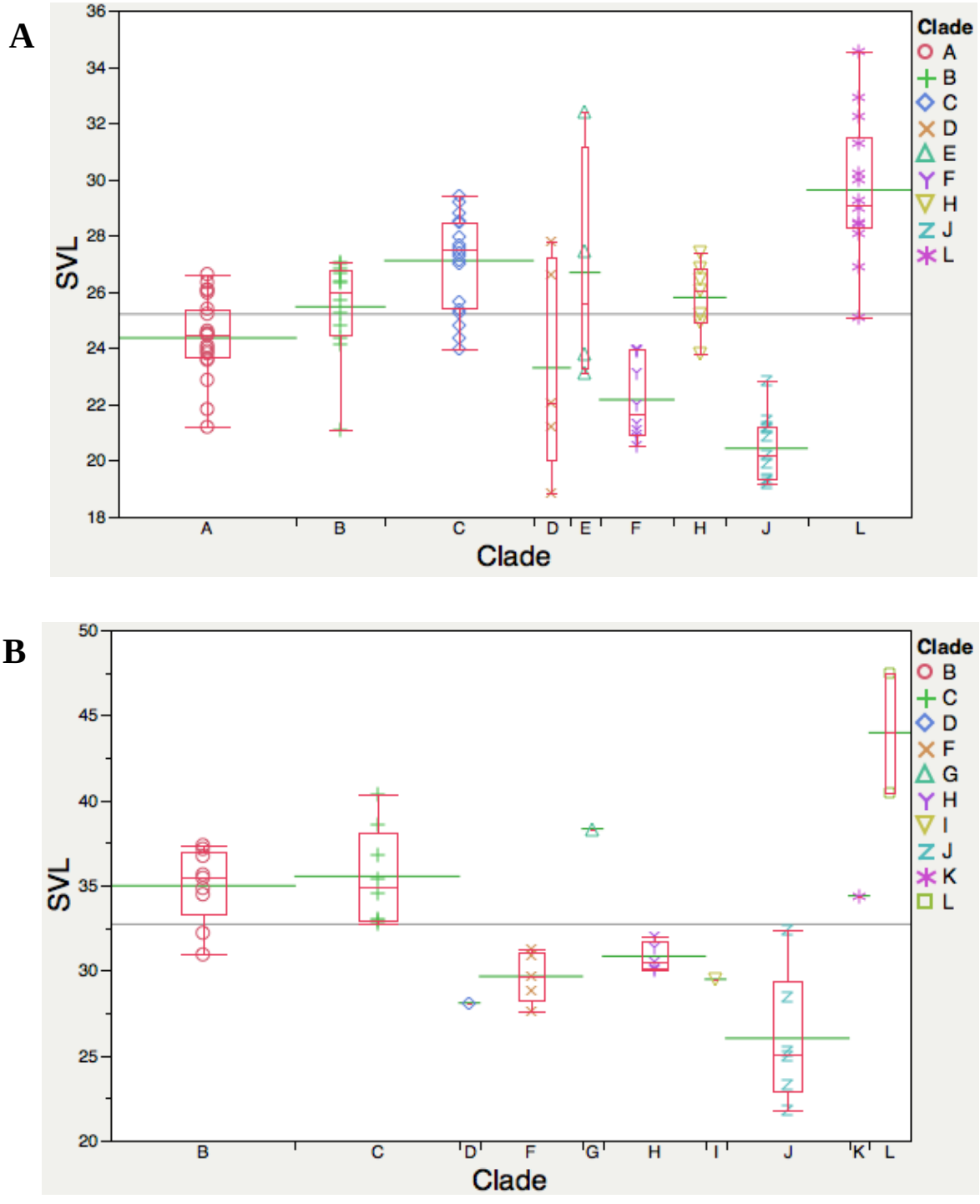

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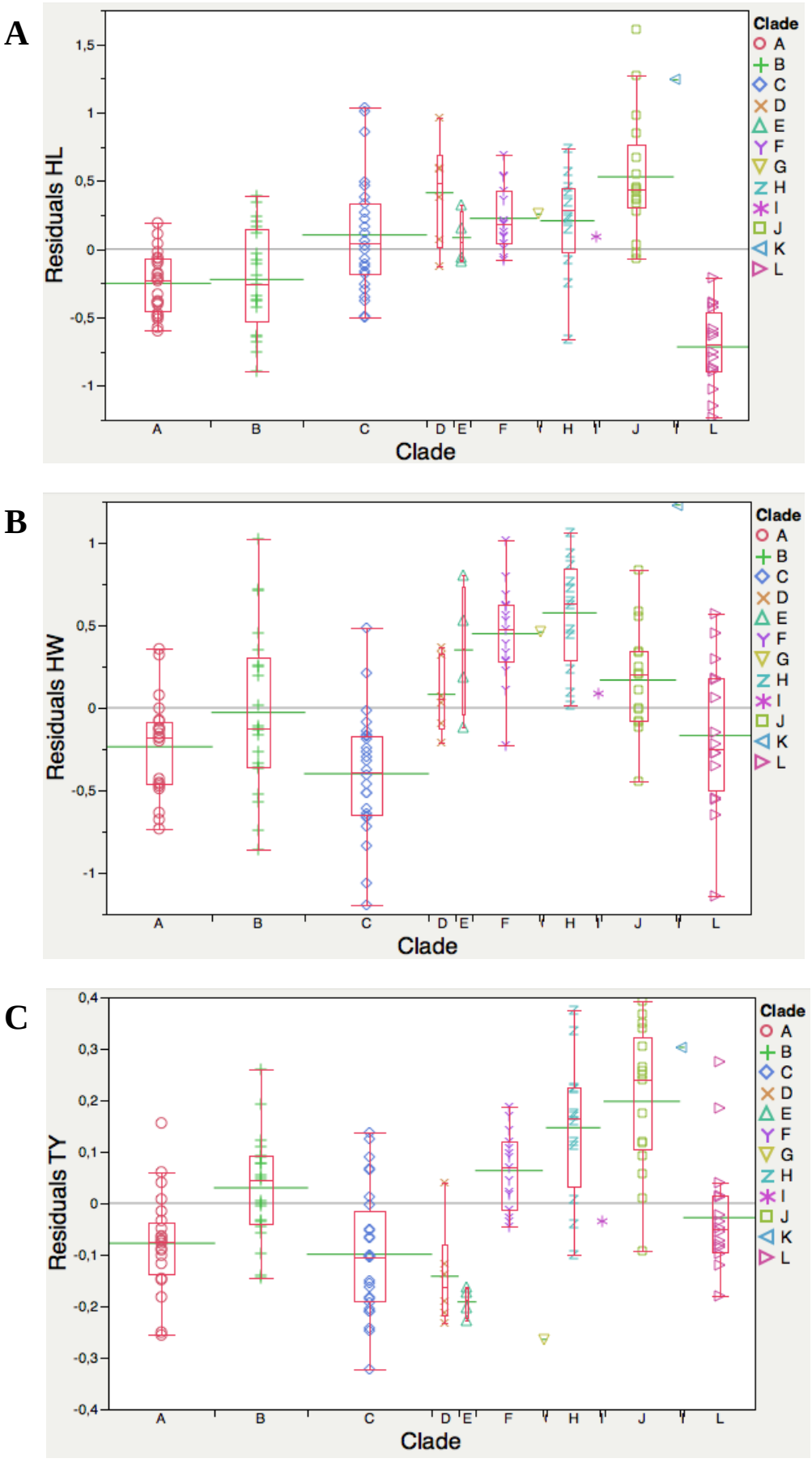
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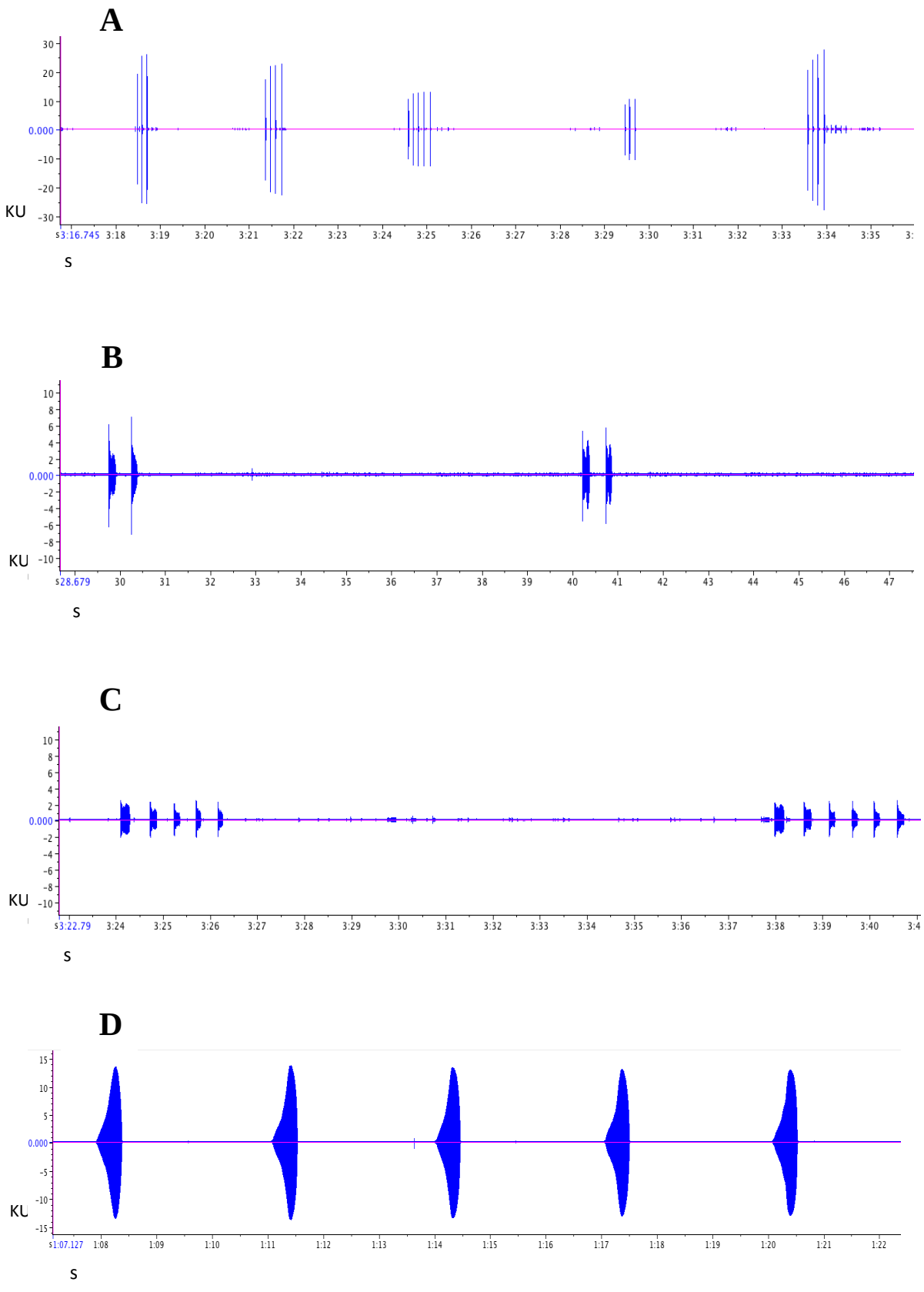
Figure 9.



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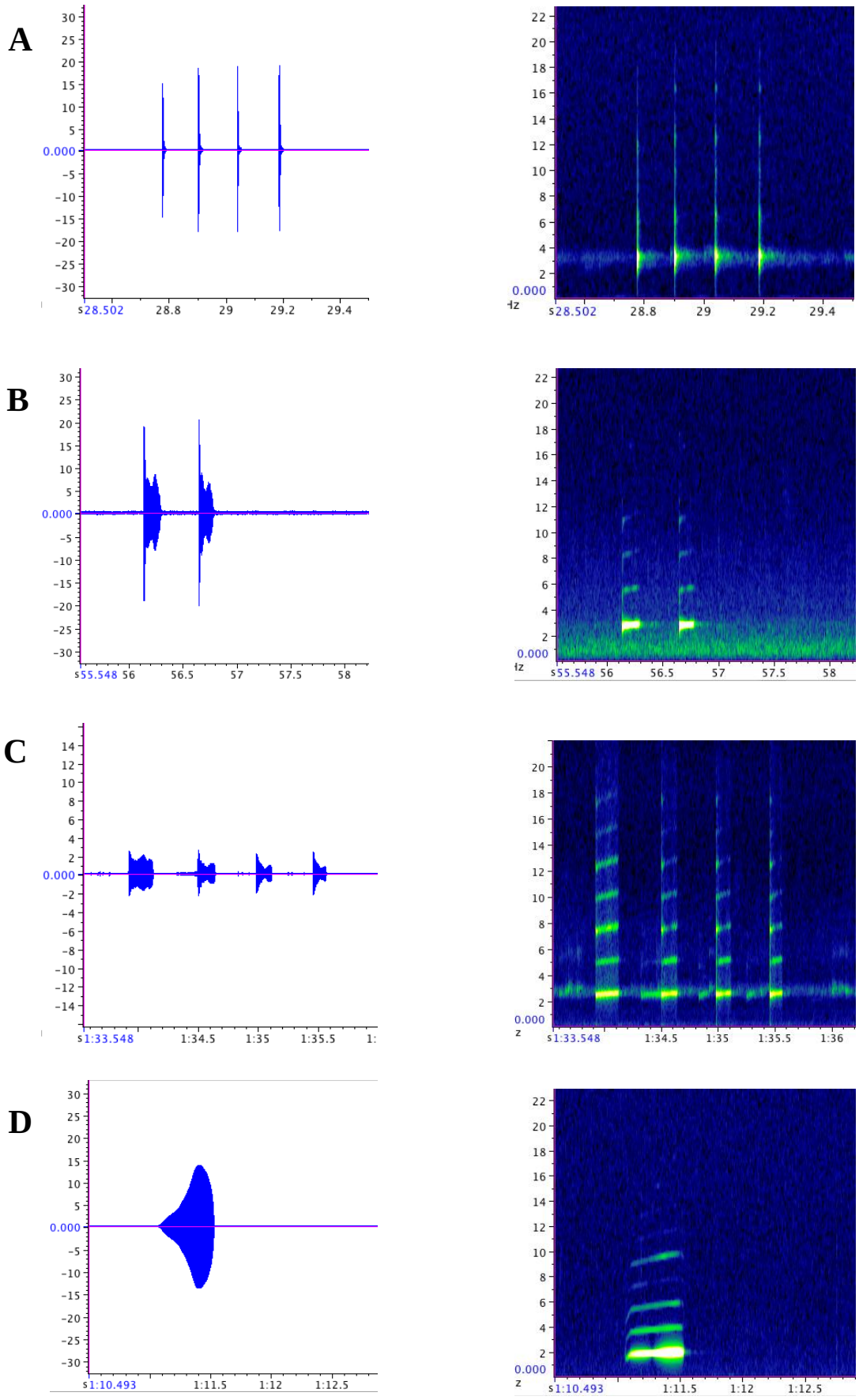
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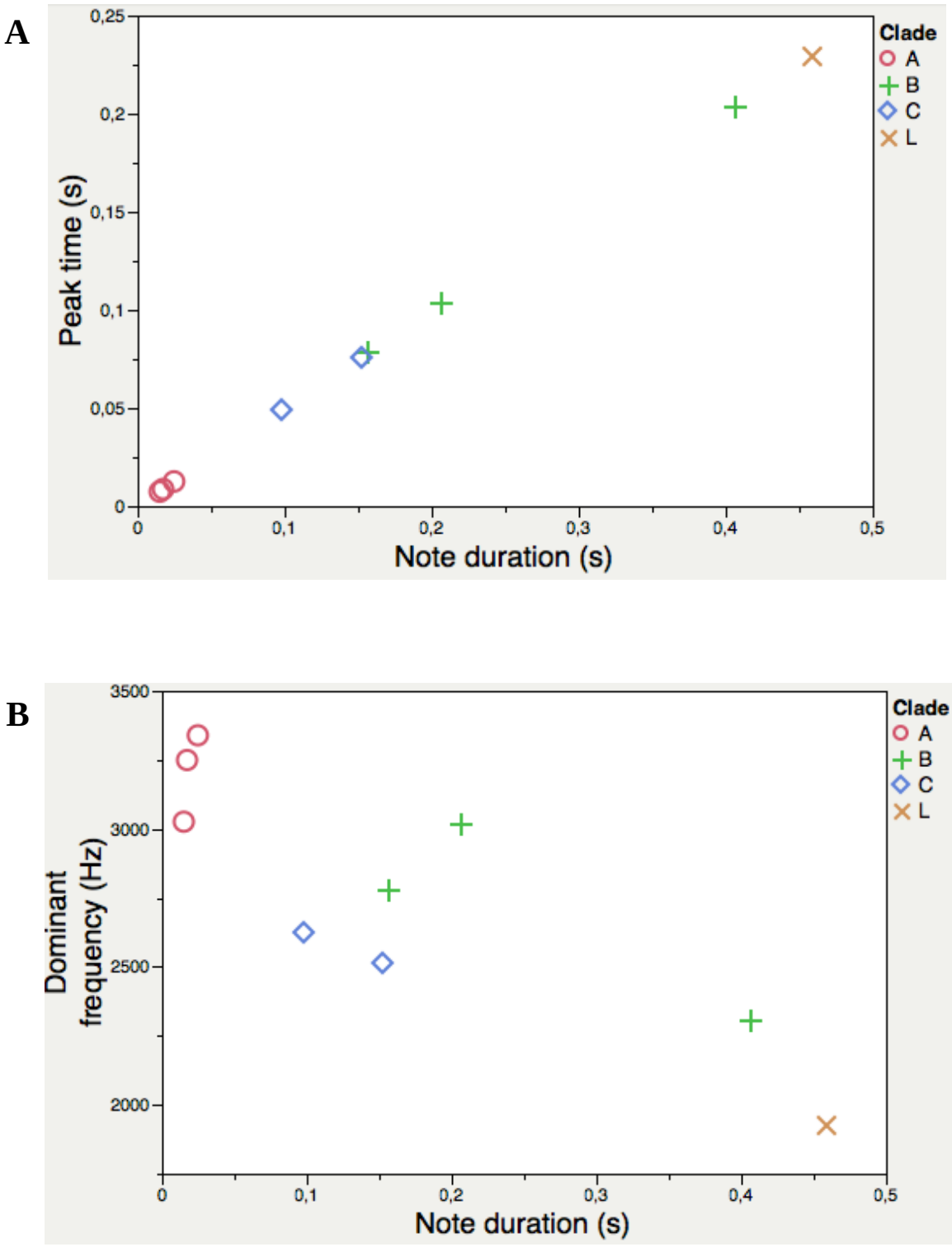
Figure 11.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Note duration (s)
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2500
2000
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0,1
0,2
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0,5
Note duration (s)
Clade
A
B
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L

Table 1. Voucher and accession numbers of DNA sequences used for phylogenetic analysis. EC is for Ecuador and PA for Panama.

Voucher	Species	Country: Province	16S	ND1	RAG1	12S
QCAZ10564	*Pristimantis pseudoacuminatus*	EC: Orellana	Pending	-	Pending	-
QCAZ11673	*P.* sp.	EC: Imbabura	Pending	Pending	Pending	-
QCAZ11737	*P. sobetes*	EC: Cotopaxi	Pending	-	Pending	-
QCAZ16365	*P. appendiculatus*	EC: Pichincha	Pending	-	Pending	-
QCAZ16428	*P. thymelensis*	EC: Napo	Pending	Pending	Pending	Pending
QCAZ16448	*P.* sp.	EC: Azuay	Pending	Pending	Pending	-
QCAZ17101	*P. malkini*	EC: Esmeraldas	Pending	Pending	Pending	Pending
QCAZ17505	*P. curtipes*	EC: Cotopaxi	Pending	Pending	Pending	-
QCAZ25105	*P. phoxocephalus*	EC: Bolívar	Pending	Pending	Pending	-
QCAZ25676	*P. quaquaversus*	EC: Pastaza	Pending	-	Pending	Pending
QCAZ25766	*P. kichwarum*	EC: Napo	Pending	-	Pending	-
QCAZ25852	*P. librarius*	EC: Napo	Pending	-	Pending	Pending
QCAZ26551	*P. rubicundus*	EC: Morona Santiago	Pending	Pending	Pending	-
QCAZ26586	*P.* sp.	EC: Carchi	Pending	-	Pending	-
QCAZ30675	*Craugastor talamancae*	PA: Bocas del Toro	Pending	Pending	Pending	-
QCAZ32785	*P.* sp.	EC: Azuay	Pending	Pending	Pending	-
QCAZ32786	*P.* sp.	EC: Azuay	Pending	Pending	Pending	-
QCAZ32790	*P.* sp.	EC: Azuay	Pending	Pending	Pending	-
QCAZ32791	*P.* sp.	EC: Azuay	Pending	Pending	Pending	-
QCAZ39568	*P. buckleyi*	EC: Napo	Pending	Pending	-	-
QCAZ39569	*P. buckleyi*	EC: Napo	Pending	Pending	-	-
QCAZ42251	*Epipedobates boulengeri*	EC: Los Ríos	Pending	Pending	Pending	-
QCAZ45129	*P.* sp.	EC: El Oro	Pending	Pending	Pending	-
QCAZ45155	*P.* sp.	EC: El Oro	Pending	Pending	Pending	-
QCAZ45170	*P.* sp.	EC: Azuay	Pending	Pending	Pending	-
QCAZ45174	*P.* sp.	EC: Azuay	Pending	Pending	Pending	-
QCAZ45178	*P.* sp.	EC: Azuay	Pending	Pending	Pending	-

Voucher	Species	Country: Province	16S	ND1	RAG1	12S
QCAZ45195	*P.* sp.	EC: Azuay	Pending	Pending	Pending	-
QCAZ45580	*P. atratus*	EC: Loja	Pending	Pending	Pending	-
QCAZ45645	*P. atratus*	EC: Loja	Pending	Pending	Pending	-
QCAZ45650	*P. versicolor*	EC: Loja	Pending	Pending	Pending	-
QCAZ45666	*P.* sp.	EC: Zamora Chinchipe	Pending	Pending	Pending	-
QCAZ45669	*P.* sp.	EC: Zamora Chinchipe	Pending	Pending	Pending	-
QCAZ46221	*P.* sp.	EC: Napo	Pending	Pending	Pending	-
QCAZ46310	*P. versicolor*	EC: Zamora Chinchipe	Pending	Pending	Pending	-
QCAZ46977	*P.* sp.	EC: Azuay	Pending	Pending	Pending	-
QCAZ46978	*P.* sp.	EC: Azuay	Pending	Pending	Pending	-
QCAZ46982	*P.* sp.	EC: Azuay	Pending	Pending	Pending	-
QCAZ46992	*P.* sp.	EC: Azuay	Pending	Pending	Pending	-
QCAZ46993	*P.* sp.	EC: Azuay	Pending	Pending	Pending	-
QCAZ47002	*P.* sp.	EC: Azuay	Pending	Pending	Pending	-
QCAZ47353	*P.* sp.	EC: Loja	Pending	Pending	Pending	-
QCAZ47397	*P. ceuthospilus*	EC: Loja	Pending	Pending	Pending	-
KU218025	*P. phoxocephalus*	EC: Chimborazo	EF493349	-	-	EF493349
KU218028	*P. pycnodermis*	EC: Morona Santiago	EF493680	-	-	EF493680
KU218030	*P. pyrrhomerus*	EC: Bolívar	EF493683	-	-	EF493683
KU218035	*P.* sp.	EC: Azuay	EF493348	-	-	EF493348
KU218052	*P. spinosus*	EC: Morona Santiago	EF493673	-	-	EF493673
KU218057	*P. unistrigatus*	EC: Imbabura	EF493387	-	EF493444	EF493387
KU218096	*P. versicolor*	EC: Zamora Chinchipe	EF493389	-	EF493431	EF493389

Table 2. Best-fit partitioning schemes and their models of molecular evolution given by PartitionFinder v.1.1.1.

Partition	Best Model	Components
1	GTR+I+G	12S, ND1 first position, 16S, noncoding ND1
2	TrN+I+G	ND1 third position
3	HKY+G	ND1 second position, noncoding RAG1
4	K80+G	RAG1 third position
5	K81uf+I+G	RAG1 first position, RAG2 second position

Table 3. Pairwise uncorrected genetic distances among clades of the *P. phoxocephalus* complex. The data shown under the diagonal correspond to the mean, standard deviation and the range -in brackets- of the distances between clades (as shown in Figs. 7 and 8). The number of individuals for each clade is shown in the first column. Data shown above the diagonal represent the number of pairwise comparison between clades. Data on the diagonal corresponds to intra-clade genetic distance.

Clade	A	B	C	D	E	F	G	H	I	J	K	L
A n=1	0											
B n=4	0.089 ± 0.001 (0.089-0.090)	0.005 ± 0.003 (0.002-0.007)										
C n=2	0.078 ± 0.004 (0.075-0.081)	0.068 ± 0.002 (0.65-0.70)	0.001									
D n=2	0.086 ± 0.000	0.067 ± 0.001 (0.66-0.68)	0.062 ± 0.006 (0.057-0.067)	0								
E n=2	0.090 ± 0.001 (0.089-0.090)	0.076 ± 0.001 (0.075-0.076)	0.069 ± 0.005 (0.064-0.074)	0.051 ± 0.000 (0.50-0.051)	0.002							
F n=2	0.077 ± 0.001 (0.077-0.078)	0.058 ± 0.001 (0.057-0.059)	0.057 ± 0.003 (0.054-0.060)	0.056 ± 0.000 (0.056-0.057)	0.060 ± 0.001 (0.059-0.060)	0.002						
G n=2	0.074 ± 0.000	0.057 ± 0.001 (0.056-0.059)	0.058 ± 0.004 (0.55-0.62)	0.058 ± 0.001 (0.057-0.059)	0.057 ± 0.001 (0.057-0.058)	0.020 ± 0.002 (0.018-0.022)	0.002					
H n=3	0.082 ± 0.002 (0.080-0.084)	0.059 ± 0.003 (0.055-0.062)	0.059 ± 0.006 (0.050-0.065)	0.057 ± 0.000	0.054 ± 0.002 (0.051-0.056)	0.037 ± 0.002 (0.035-0.039)	0.031 ± 0.002 (0.029-0.032)	0				
I n=1	0.086	0.067 ± 0.000	0.063 ± 0.001 (0.062-0.063)	0.064 ± 0.000	0.070 ± 0.001 (0.069-0.070)	0.048 ± 0.000	0.052 ± 0.000	0.051 ± 0.000	0			
J n=3	0.086 ± 0.002 (0.085-0.088)	0.074 ± 0.001 (0.073-0.076)	0.069 ± 0.001 (0.068-0.071)	0.072 ± 0.001 (0.071-0.072)	0.072 ± 0.001 (0.070-0.073)	0.056 ± 0.001 (0.055-0.057)	0.057 ± 0.001 (0.56-0.058)	0.057 ± 0.002 (0.057-0.059)	0.036 ± 0.001 (0.036-0.037)	0.004 ± 0.002 (0.002-0.005)		
K n=1	0.084	0.063 ± 0.002 (0.061-0.064)	0.061 ± 0.000	0.062 ± 0.000	0.069 ± 0.001 (0.068-0.069)	0.048 ± 0.000	0.051 ± 0.001 (0.051-0.052)	0.055 ± 0.002 (0.053-0.056)	0.04	0.049 ± 0.001 (0.048-0.050)	0	
L n=5	0.081 ± 0.001 (0.080-0.082)	0.055 ± 0.002 (0.053-0.057)	0.057 ± 0.002 (0.055-0.060)	0.054 ± 0.001 (0.053-0.055)	0.063 ± 0.001 (0.061-0.064)	0.043 ± 0.001 (0.042-0.044)	0.045 ± 0.001 (0.043-0.047)	0.049 ± 0.001 (0.047-0.050)	0.036 ± 0.000	0.047 ± 0.001 (0.045-0.048)	0.032 ± 0.001 (0.032-0.033)	0.002

Table 4. Diagnostic characters of candidate species within the *Pristimantis phoxocephalus* complex.

	Males traits			**Snout shape**		**Fingers**
	Vocal Sac	**Vocal slits**	**Nuptial pad**	**Dorsal view**	**Lateral view**	**Discs**
A	Present. Large	Present	Present	Acuminate	Moderately long, barely protruding	Expanded, truncate.
B	Present. Small to median	Present	Present	Acuminate with vertical keel	Moderate in length, protruding	Broadly expanded, elliptic to truncate.
C	Present. Large	Present	Present	Acuminate with vertical keel	Moderate in length, protruding	Broadly expanded, rounded. Short and sturdy fingers.
D	Present. Small	Present	Absent	Acuminate	Moderate in length, barely protruding	Expanded, elliptic.
E	Present. Median	Present	Present	Subacuminate with small papilla	Moderate in length, rounded	Expanded, elliptic to truncate.
F	Present. Large	Present	Present	Subacuminate	Short, rounded to barely protruding	Expanded, elliptic to truncate.
G	-	-	-	Rounded with small papilla	Short, rounded	Broadly expanded, elliptic.
H	Present. Large	Present	Present	Rounded to subacuminate	Short, rounded	Not expanded to slightly expanded, rounded.
I	-	-	-	Rounded with papilla	Moderate in length, rounded with papilla	Broadly expanded, rounded.
J	Absent	Absent	Present	Subacuminate	Moderate in length, rounded	Slightly expanded, rounded.
K	-	-	-	Rounded	Short, rounded	Slightly expanded, rounded.
L	Present. Median	Present	Present	Acuminate with vertical keel	Moderate in length, protruding	Broadly expanded, elliptic.

	Texture of skin	Eyelid Tubercles	Postrictal Tubercles	Heel Tubercles	Dermal folds
A	Dorsum shagreen with minute round tubercles, as well as limbs and flanks. Venter coarsely aerolate; vocal sac equal or less.	Minute and round as the rest of the dorsum.	2 large, subconical	Granular with one protruding tubercle of median elevation	Dorsolateral folds absent. Supratympanic folds thin and inconspicuous. Discoidal fold present.
B	Dorsum shagreen with minute round tubercles, as well as limbs and flanks. Venter aerolate; vocal sac less aerolate.	Same texture of dorsum with higher tubercles in posterior region	2 medium, rounded to subconical, low tubercles	One small, subconical, middle elevated tubercle	Dorsolateral folds always present, inconspicuous, looking like wrinkles. Thick and inconspicuous supratympanic folds. Discoidal fold absent.
C	Dorsum shagreen with small, round tubercles as well as limbs. Flanks have larger tubercles. Venter coarsely aerolate.	Same texture of dorsum with higher tubercles in posterior region	2 conspicuous, subconical.	Granular or apparently absent.	Dorsolateral folds absent. Supratympanic folds slightly prominent. Discoidal fold present.
D	Dorsum shagreen with minute round tubercles forming low ridges. Venter aerolate.	Present	2 large and low, almost unperceivable	A calcar large, conical and very prominent.	Dorsolateral folds complete, thick and conspicuous. Supratympanic folds thick and conspicuous, it doesn't hide superior and posterior border of tympanum. Discoidal fold absent
E	Dorsum shagreen with minute round tubercles forming low ridges. Flanks with pustules. Venter granular.	Absent	2 large and low, almost unperceivable	One small, subconical, middle elevated tubercle	Pustules on flanks are often aligned and form the dorsolateral folds. Supratympanic fold very thick and conspicuous. Discoidal fold absent.
F	Dorsum shagreen with minute low round tubercles. Flanks have pustules. Venter coarsely aerolate.	Absent	2 large, rounded, low to middle elevation	One small, rounded and middle elevated tubercle or apparently absent.	Dorsolateral folds absent. Thick supratympanic folds. Discoidal fold present.
G	Dorsum shagreen with medium, round, low tubercles. Flanks with higher tubercles Venter coarsely aerolate.	Same texture of dorsum.	2 medium, subconical	Granular with one protruding tubercle of median elevation	Dorsolateral folds absent. Thick supratympanic folds. Discoidal fold absent.
H	Dorsum smooth forming low ridges. Flanks have pustules. Venter coarsely aerolate.	Absent	2 large, rounded, low to middle elevation	One small, rounded and middle elevated tubercle or apparently absent.	Dorsolateral folds absent. Thick supratympanic folds. Discoidal and thoracic fold present.
I	Shagreen with small, conical, middle elevated tubercles.	Large, conical, elevated.	2 not prominent	Same texture of dorsum with a prominent tubercle.	Dorsolateral folds absent. Inconspicuous supratympanic folds. Discoidal fold absent.
J	Dorsum tuberculate with minute, round tubercles and scattered medium ones.	Small, subconical, middle elevated.	1-4 middle elevated.	Granular with one protruding tubercle of median elevation	Dorsolateral folds absent. Thick supratympanic folds, posterior part formed by a row of tubercles.
K	Dorsum smooth forming low ridges. Flanks have pustules. Venter coarsely aerolate.	Absent	2 large, rounded, low to middle elevation	One small, rounded and middle elevated tubercle or apparently absent.	Dorsolateral folds absent. Thick supratympanic folds. Discoidal and thoracic fold present. Discoidal folds present.
L	Dorsum tuberculate with small rounded middle elevated tubercles and large, rounded, middle elevated ones. Venter coarsely aerolate.	Present, small rounded, middle elevated.	2-3 conspicuous	Granular with one protruding tubercle of median elevation	Dorsolateral folds present, sometimes just as a row of tubercles. Supratympanic folds conspicuous. Thoracic fold present.

	Coloration			
	Dorsal	**Ventral**	**Posterior surfaces of thighs**	**Head markings**
A	Dorsum gray to pale brown usually with small brown flecks or streaks. A pale middorsal stripe may be present.	Venter cream with or without minute dark flecks; abdominal muscles visible.	Dark, with large and pale spots. Groin with the same pattern.	Supratympanic stripe present; labial bars, interorbital bars or interorbital stripes may be present.
B	Cream to pale brown. Sometimes a pale middorsal band or stripe may be present, otherwise they bear a W-shaped mark on the scapula.	Cream with or without dark marks.	Cream to pale brown with light stripes or medium to small spots. Groin and axillae with the same pattern	Marked supratympanic stripe always present; canthal stripe less marked; facial mask and labial bars may be present.
C	Various dorsal patterns; cream to brown with or without dark flecks, middorsal bar or stripe. Two pale blotches or scattered white dots may be present.	Cream with or without dark marks.	Cream to pale brown with light stripes or medium to small spots. Groin and axillae with the same pattern.	Poorly defined supratympanic stripe present; canthal stripe, labial bars, facial mask or interorbital bar may be present.
D	Cream to gray with scattered little dark spots frequently arranged in a longitudinal pattern.	White to cream with scattered Little dark spots.	White to cream with thick and black reticulations.	Marked supratympanic and canthal stripes always presents.
E	Cream to brown with dark flecks or spots.	Brown mottled with cream.	Dark brown with small spots. Groin, axillae, hidden surface of shank and tarsus with the same pattern.	Marked supratympanic stripe always present; canthal stripe and labial bars may be present.
F	Gray to brown; various patterns. Characteristic scattered black spots frequently arranged symmetrically.	Cream, frequently with reddish reticulations convergent with areolation pattern.	Brown with small-medium white spots. Groin and axillae with the same pattern.	Supratympanic stripe present; canthal stripe, interorbital stripe or facial mask may be present.
G	Cream without a distinctive pattern	Cream with dark marks.	Brown with medium white spots. Groin with the same pattern.	Supratympanic stripe, interorbital bar and facial mask present
H	Gray to brown; various patterns. Characteristic scattered black spots frequently arranged symmetrically.	Cream, frequently with reddish reticulations convergent with areolation pattern.	Brown with medium-large white spots. Groin and axillae with the same pattern.	Supratympanic stripe present; canthal stripe, interorbital stripe or facial mask may be present.
I	Light gray with marked dark chevrons.	White with dark markings.	Banding pattern.	Supratympanic, canthal stripe and interorbital bar present.
J	Gray to brown with variegated or simple pattern. Variegated patterns the most common. Flanks gray to brown with light diagonal reticulations.	Cream with or without dark marks.	Brown with small and pale spots. Hidden surface of thighs with small pale spots.	Marked supratympanic and canthal stripes always presents. Labial bars always present.
K	Gray to brown; various patterns. Characteristic scattered black spots frequently arranged symmetrically.	Cream, frequently with reddish reticulations convergent with areolation pattern.	Brown with medium-large white spots. Groin and axillae with the same pattern.	Supratympanic stripe present; canthal stripe, interorbital stripe or facial mask may be present.
L	Gray to brown without a distinctive pattern.	Cream with or without dark marks.	Brown with pale stripes or small spots. Groin, axillae, hidden surface of shank and tarsus with the same pattern.	Marked supratympanic and canthal stripes, continuous, always present; interorbital bar frequently present; labial bars may be present.

Table 5. Descriptive statistics for morphometric measurements of males of the *P. phoxocephalus* complex. In each cell the mean, standard deviation and range (in parenthesis) are given. Morphometric variables are: SVL= snout-vent length; HL= head length; HW= head width; ED= eye diameter; TY= tympanum diameter. All measurements are expressed in millimeters. n= sample size.

Clade	SVL	HL	HW	TY
A n=24	24.35 ± 1.37 (21.14-26.34)	8.69 ± 0.42 (8.04-9.34)	8.67 ± 0.60 (7.15-9.47)	1.24 ± 0.13 (0.93-1.44)
B n=12	25.53 ± 1.53 (21.91-26.99)	8.79 ± 0.59 (7.38-9.47)	9.09 ± 0.68 (7.53-9.94)	1.40 ± 0.07 (1.27-1.50)
C n=21	26.83 ± 1.82 (23.12-29.42)	9.62 ± 0.51 (8.71-10.41)	9.44 ± 0.48 (8.44-10.3)	1.29 ± 0.13 (1.13-1.49)
D n=5	23.28 ± 3.81 (18.66-27.77)	8.95 ± 1.32 (7.50-10.47)	8.61 ± 1.33 (6.68-9.99)	1.12 ± 0.16 (0.92-1.27)
E n=4	26.69 ± 4.25 (23.13-32.38)	9.66 ± 1.11 (8.52-11.07)	10.13 ± 1.58 (8.71-12.39)	1.24 ± 0.18 (1.09-1.50)
F n=10	22.19 ± 1.50 (20.44-24.28)	8.52 ± 0.55 (7.80-9.35)	8.64 ± 0.55 (7.88-9.38)	1.27 ± 0.11 (1.13-.1.37)
H n=7	25.80 ± 1.24 (23.77-27.38)	9.36 ± 0.33 (8.84-9.74)	9.96 ± 0.43 (9.51-10.64)	1.46 ± 0.13 (1.30-1.64)
J n=11	20.43 ± 1.16 (19.17-22.81)	8.18 ± 0.52 (7.39-9.12)	7.67 ± 0.50 (7.04-8.49)	1.31 ± 0.18 (0.99-1.64)
L n=14	29.62 ± 2.49 (25.06-34.53)	9.61 ± 0.66 (8.50-10.69)	10.57 ± 0.87 (9.00-12.09)	1.54 ± 0.15 (1.37-1.77)

Table 6. Descriptive statistics for morphometric measurements of females of the *P. phoxocephalus* complex. In each cell the mean, standard deviation and range (in parenthesis) are given. All measurements are expressed in millimeters. n=sample size.

Clade	SVL	HL	HW	TY
B n=9	34.97 ± 2.19 (30.94-37.36)	11.91 ± 0.56 (10.97-12.77)	13.1 ± 0.65 (12.05-13.91)	1.88 ± 0.16 (1.77-2.2)
C n=10	35.10 ± 2.65 (32.71-40.32)	12.49 ± 0.91 (11.44-13.84)	12.7 ± 0.80 (11.90-14.20)	1.85 ± 0.18 (1.63-2.00)
D n=1	28.09	10.92	10.68	1.38
F n=5	29.65 ± 1.51 (27.64-31.29)	10.59 ± 0.15 (10.50-10.85)	11.3 ± 0.67 (10.79-12.48)	1.66 ± 0.12 (1.45-1.76)
G n=1	38.32	13	14.46	1.72
H n=5	30.83 ± 0.85 (30.04-32.03)	11.15 ± 0.40 (10.67-11.59)	12.02 ± 0.46 (11.46-12.67)	1.88 ± 0.14 (1.71-2.06)
I n=1	29.48	10.43	10.9	1.53
J n=6	26.01 ± 3.83 (21.80-32.39)	10.37 ± 1.36 (9.03-11.65)	9.84 ± 1.25 (8.58-8.42)	1.65 ± 0.24 (1.44-2.07)
K n=1	34.38	12.91	13.81	2.1
L n=2	43.97 ± 5.00 (40.43-47.50)	13.89 ± 1.10 (13.11-14.66)	16.55 ± 1.72 (15.33-17.76)	2.35 ± 0.01 (2.34-2.36)

Table 7. Descriptive statistics for call variables of the clades A, B, C and L of *P. phoxocephalus* complex. The data shown correspond to the mean, standard deviation and the range -in brackets- of the variables for each clade. "n" is for the number of recordings analyzed. Specimens from whom advertisement calls were recorded are: QCAZ47397, QCAZ47398, QCAZ47487, QCAZ25105, QCAZ45178, QCAZ46977, QCAZ46980, QCAZ46981 and one recording without a collection number from Cashca Totoras-Bolívar.

Clade	Notes per call	Note duration (s)	Interval between notes (s)	Peak time (s)	Dominant frequency (Hz)
A n=3	1-7	0.019 ± 0.005 (0.015-0.024)	0.103 ± 0.002 (0.101-0.105)	0.010 ± 0.003 (0.007-0.013)	3204.84 ± 161.29 (3025.92-3339.07)
B n=3	1-2	0.257 ± 0.132 (0.157-0.407)	0.297 ± 0.088 (0.234-0.360)	0.128 ± 0.066 (0.76-0.103)	2696.91 ± 362.27 (2301.58-3013.01)
C n=2	2-6	0.125 ± 0.038 (0.098-0.152)	0.339 ± 0.038 (0.312-0.366)	0.063 ± 0.019 (0.049-0.076)	2569.65 ± 21.21 (2512.22-2627.07)
L n=1	1	0.458	-	0.229	1926.58

Suplemmentary file 1. List of individuals used for the study. L.M. is for the number of locality traced on the map (Fig. 1). Latitude and longitude are expressed according to the Geographic Coordinate System. Altitude is measured as meters above sea level.

QCAZ	Clade	Province	Locality	Latitude	Longitude	Altitude	L.M.
10499	A	Azuay	Valle de Yunguilla, Chalcápac	-3.23	-79.2	1550	20
10500	A	Azuay	Valle de Yunguilla, Chalcápac	-3.23	-79.2	1550	20
10501	A	Azuay	Valle de Yunguilla, Chalcápac	-3.23	-79.2	1550	20
10502	A	Azuay	Valle de Yunguilla, Chalcápac	-3.23	-79.2	1550	20
10503	A	Azuay	Valle de Yunguilla, Chalcápac	-3.23	-79.2	1550	20
10504	A	Azuay	Valle de Yunguilla, Chalcápac	-3.23	-79.2	1550	20
10505	A	Loja	Vilcabamba, Río Uchima	-4.26	-79.22	1480	20
47397	A	Loja	Ciudad de Celica a 2 km vía Alamor	-4.1	-79.98	2101	23
47398	A	Loja	Ciudad de Celica a 2 km vía Alamor	-4.1	-79.98	2101	23
47405	A	Loja	Ciudad de Celica a 2 km vía Alamor	-4.1	-79.98	2101	23
47406	A	Loja	Ciudad de Celica a 2 km vía Alamor	-4.1	-79.98	2101	23
47407	A	Loja	Ciudad de Celica a 2 km vía Alamor	-4.1	-79.98	2101	23
47408	A	Loja	Ciudad de Celica a 2 km vía Alamor	-4.1	-79.98	2101	23
47409	A	Loja	Ciudad de Celica a 2 km vía Alamor	-4.1	-79.98	2101	23
47410	A	Loja	Ciudad de Celica a 2 km vía Alamor	-4.1	-79.98	2101	23
47411	A	Loja	Ciudad de Celica a 2 km vía Alamor	-4.1	-79.98	2101	23
47412	A	Loja	Ciudad de Celica a 2 km vía Alamor	-4.1	-79.98	2101	23
47413	A	Loja	Ciudad de Celica a 2 km vía Alamor	-4.1	-79.98	2101	23
47414	A	Loja	Ciudad de Celica a 2 km vía Alamor	-4.1	-79.98	2101	23
47415	A	Loja	Ciudad de Celica a 2 km vía Alamor	-4.1	-79.98	2101	23
47416	A	Loja	Ciudad de Celica a 2 km vía Alamor	-4.1	-79.98	2101	23
47417	A	Loja	Ciudad de Celica a 2 km vía Alamor	-4.1	-79.98	2101	23

QCAZ	Clade	Province	Locality	Latitude	Longitude	Altitude	L.M.
47418	A	Loja	Ciudad de Celica a 2 km vía Alamor	-4.1	-79.98	2101	23
47419	A	Loja	Ciudad de Celica a 2 km vía Alamor	-4.1	-79.98	2101	23
47487	A	Loja	Ciudad de Celica a 2 km vía Alamor	-4.1	-79.98	2101	23
47500	A	Loja	Ciudad de Celica a 2 km vía Alamor	-4.1	-79.98	2101	23
47501	A	Loja	Ciudad de Celica a 2 km vía Alamor	-4.1	-79.98	2101	23
47502	A	Loja	Ciudad de Celica a 2 km vía Alamor	-4.1	-79.98	2101	23
47503	A	Loja	Ciudad de Celica a 2 km vía Alamor	-4.1	-79.98	2101	23
47504	A	Loja	Ciudad de Celica a 2 km vía Alamor	-4.1	-79.98	2101	23
45170	B	Azuay	San Antonio, finca del Sr Morocho. Limita con Reserva Cajas	-2.88	-79.41	2099	7
45178	B	Azuay	San Antonio, finca del Sr Morocho. Limita con Reserva Cajas	-2.88	-79.41	2099	7
45179	B	Azuay	San Antonio, finca del Sr Morocho. Limita con Reserva Cajas	-2.88	-79.41	2099	7
45186	B	Azuay	San Antonio, finca del Sr Morocho. Limita con Reserva Cajas	-2.88	-79.41	2099	7
45187	B	Azuay	San Antonio, finca del Sr Morocho. Limita con Reserva Cajas	-2.88	-79.41	2099	7
45188	B	Azuay	San Antonio, finca del Sr Morocho. Limita con Reserva Cajas	-2.88	-79.41	2099	7
45189	B	Azuay	San Antonio, finca del Sr Morocho. Limita con Reserva Cajas	-2.88	-79.41	2099	7
45196	B	Azuay	San Antonio, finca del Sr Morocho. Limita con Reserva Cajas	-2.86	-79.38	2900	7
45199	B	Azuay	San Antonio, finca del Sr Morocho. Limita con Reserva Cajas	-2.86	-79.38	2900	7
45201	B	Azuay	San Antonio, pueblo de San Antonio, Río Malacatos	-2.91	-79.41	1800	7
45202	B	Azuay	San Antonio, pueblo de San Antonio, Río Malacatos	-2.91	-79.41	1800	7
45203	B	Azuay	San Antonio, pueblo de San Antonio, Río Malacatos	-2.91	-79.41	1800	7
45204	B	Azuay	San Antonio, pueblo de San Antonio, Río Malacatos	-2.91	-79.41	1800	7
45205	B	Azuay	San Antonio, pueblo de San Antonio, Río Malacatos	-2.91	-79.41	1800	7
45206	B	Azuay	San Antonio, pueblo de San Antonio, Río Malacatos	-2.91	-79.41	1800	7
45207	B	Azuay	San Antonio, pueblo de San Antonio, Río Malacatos	-2.91	-79.41	1800	7
45208	B	Azuay	San Antonio, pueblo de San Antonio, Río Malacatos	-2.91	-79.41	1800	7
45209	B	Azuay	San Antonio, pueblo de San Antonio, Río Malacatos	-2.91	-79.41	1800	7

QCAZ	Clade	Province	Locality	Latitude	Longitude	Altitude	L.M.
45211	B	Azuay	San Antonio, pueblo de San Antonio, Río Malacatos	-2.91	-79.41	1800	7
45212	B	Azuay	San Antonio, pueblo de San Antonio, Río Malacatos	-2.91	-79.41	1800	7
46955	B	Azuay	Sector Río Chipla	-2.75	-79.41	2500	6
46977	B	Azuay	Molleturo, Río Zadracay	-2.74	-79.41	2674	6
46978	B	Azuay	Molleturo, Río Zadracay	-2.74	-79.41	2674	6
46979	B	Azuay	Molleturo, Río Zadracay	-2.74	-79.41	2674	6
46980	B	Azuay	Molleturo, Río Zadracay	-2.74	-79.41	2674	6
554	C	Cotopaxi	Pilaló	-0.94	-78.99	-	2
1021	C	Bolívar	Bosque Protector Cashca Totoras	-1.71	-78.98	3000	4
1022	C	Bolívar	Bosque Protector Cashca Totoras	-1.71	-78.98	3000	4
1024	C	Bolívar	Bosque Protector Cashca Totoras	-1.71	-78.98	3000	4
3022	C	Chimborazo	A 70 km oeste de Riobamba, vía Papallacta	-1.96	-78.98	2520	5
3023	C	Chimborazo	A 70 km oeste de Riobamba, vía Papallacta	-1.96	-78.98	2520	5
12580	C	Bolívar	Bosque Protector Cashca Totoras	-1.72	-78.96	3200	4
14704	C	Bolívar	Bosque Protector Cashca Totoras	-1.71	-78.98	-	4
14705	C	Bolívar	Bosque Protector Cashca Totoras	-1.71	-78.98	-	4
16729	C	Bolívar	Bosque Protector Cashca Totoras	-1.71	-78.98	-	4
16730	C	Bolívar	Bosque Protector Cashca Totoras	-1.71	-78.98	-	4
16834	C	Bolívar	Bosque Protector Cashca Totoras	-1.71	-78.98	3000	4
16835	C	Bolívar	Bosque Protector Cashca Totoras	-1.71	-78.98	3000	4
16836	C	Bolívar	Bosque Protector Cashca Totoras	-1.71	-78.98	3000	4
16916	C	Bolívar	Bosque Protector Cashca Totoras	-1.71	-78.98	3000	4
16917	C	Bolívar	Bosque Protector Cashca Totoras	-1.71	-78.98	3000	4
16918	C	Bolívar	Bosque Protector Cashca Totoras	-1.71	-78.98	3000	4
16919	C	Bolívar	Bosque Protector Cashca Totoras	-1.71	-78.98	3000	4
16920	C	Bolívar	Bosque Protector Cashca Totoras	-1.71	-78.98	3000	4

QCAZ	Clade	Province	Locality	Latitude	Longitude	Altitude	L.M.
16921	C	Bolívar	Bosque Protector Cashca Totoras	-1.71	-78.98	3000	4
16922	C	Bolívar	Bosque Protector Cashca Totoras	-1.71	-78.98	3000	4
17602	C	Cotopaxi	Alrededor de Pilaló y Río Pilaló	-0.95	-78.99	-	2
25105	C	Bolívar	Bosque Protector Cashca Totoras	-1.72	-78.97	-	4
25122	C	Chimborazo	Bosque Protector Cashca Totoras	-1.72	-78.97	-	4
25124	C	Chimborazo	Bosque Protector Cashca Totoras	-1.72	-78.97	2960	4
25125	C	Bolívar	Bosque Protector Cashca Totoras	-1.71	-78.98	2960	4
25127	C	Chimborazo	Bosque Protector Cashca Totoras	-1.72	-78.97	2960	4
25128	C	Chimborazo	Bosque Protector Cashca Totoras	-1.72	-78.97	2960	4
25134	C	Chimborazo	Bosque Protector Cashca Totoras	-1.72	-78.97	2960	4
25135	C	Chimborazo	Bosque Protector Cashca Totoras	-1.72	-78.97	2960	4
29752	C	Bolívar	Bosque Protector Cashca Totoras	-1.72	-78.97	2900	4
31502	C	Bolívar	Bosque Protector Cashca Totoras	-1.73	-78.98	2900	4
31505	C	Bolívar	Bosque Protector Cashca Totoras	-1.73	-78.98	2900	4
32039	C	Bolívar	Bosque Protector Cashca Totoras	-1.73	-78.98	3000	4
32040	C	Bolívar	Bosque Protector Cashca Totoras	-1.73	-78.98	3000	4
36845	C	Cotopaxi	Río Pilaló	-0.95	-78.99	-	2
36846	C	Cotopaxi	Río Pilaló	-0.95	-78.99	-	2
36847	C	Cotopaxi	Río Pilaló	-0.95	-78.99	-	2
36850	C	Cotopaxi	Río Pilaló	-0.95	-78.99	-	2
42596	C	Bolívar	Bosque Protector Cashca Totoras	-1.71	-78.98	-	4
49509	C	Bolívar	San Vicente	-1.71	-79.02	-	4
49511	C	Bolívar	San Vicente	-1.71	-79.02	-	4
51655	C	Cotopaxi	Sigchos	-0.7	-78.89	-	1
20991	D	Loja	Abra de Zamora Loja-Zamora	-4	-79.14	2800	17
45465	D	Loja	Parque Nacional Podocarpus, Cajanuma	-4.12	-79.17	2812	18

QCAZ	Clade	Province	Locality	Latitude	Longitude	Altitude	L.M.
45576	D	Loja	San Sebastián, Sector Cajanuma	-4.09	-79.2	2523	18
45579	D	Loja	San Sebastián, Sector Cajanuma	-4.09	-79.2	2523	18
45580	D	Loja	San Sebastián, Sector Cajanuma	-4.09	-79.2	2523	18
45613	D	Zamora Chinchipe	Reserva Tapichalaca, Estación Científica	-4.49	-79.12	2567	22
45645	D	Loja	Parque Nacional Podocarpus, Cajanuma	-4.12	-79.17	2812	18
45654	D	Zamora Chinchipe	Reserva Tapichalaca, Estación Científica	-4.49	-79.12	2567	22
54342	D	Loja	Shucos	-3.93	-79.22	-	16
54344	D	Loja	Shucos	-3.93	-79.22	-	16
54348	D	Loja	Shucos	-3.93	-79.22	-	16
45677	E	Zamora Chinchipe	Reserva Tapichalaca	-4.47	-79.19	-	21
45664	E	Zamora Chinchipe	Reserva Tapichalaca	-4.47	-79.19	-	21
45665	E	Zamora Chinchipe	Reserva Tapichalaca	-4.47	-79.19	-	21
45666	E	Zamora Chinchipe	Reserva Tapichalaca	-4.47	-79.19	-	21
45667	E	Zamora Chinchipe	Reserva Tapichalaca	-4.47	-79.19	-	21
45668	E	Zamora Chinchipe	Reserva Tapichalaca	-4.47	-79.19	-	21
45669	E	Zamora Chinchipe	Reserva Tapichalaca	-4.47	-79.19	-	21
45670	E	Zamora Chinchipe	Reserva Tapichalaca	-4.47	-79.19	-	21
47516	E	Zamora Chinchipe	Reserva Tapichalaca	-4.47	-79.19	-	21
47730	E	Zamora Chinchipe	Reserva Tapichalaca	-4.47	-79.19	-	21
47731	E	Zamora Chinchipe	Reserva Tapichalaca	-4.47	-79.19	-	21
47732	E	Zamora Chinchipe	Reserva Tapichalaca	-4.47	-79.19	-	21
47733	E	Zamora Chinchipe	Reserva Tapichalaca	-4.47	-79.19	-	21
47515	E	Zamora Chinchipe	Reserva Tapichalaca	-4.47	-79.19	-	21
3451	F	Azuay	Límite provincial Azuay con Morona Santiago	-3.19	-78.8	-	13
6351	F	Azuay	Carretera Sigsig-Chiguinza	-3.08	-78.72	3900	12
16447	F	Azuay	Patacocha Gualaceo-Macas	-3.12	-79.07	-	10

QCAZ	Clade	Province	Locality	Latitude	Longitude	Altitude	L.M.
16448	F	Azuay	Patacocha Gualaceo-Macas	-3.12	-79.07	-	10
16449	F	Azuay	Patacocha Gualaceo-Macas	-3.12	-79.07	-	10
16452	F	Azuay	Patacocha Gualaceo-Macas	-3.12	-79.07	-	10
23929	F	Morona Santiago	Vía Gualaquiza-Sig-Sig	-3.18	-78.8	3525	13
23930	F	Morona Santiago	Vía Gualaquiza-Sig-Sig	-3.18	-78.8	3525	13
23931	F	Morona Santiago	Vía Gualaquiza-Sig-Sig	-3.18	-78.8	3525	13
23932	F	Morona Santiago	Vía Gualaquiza-Sig-Sig	-3.18	-78.8	3525	13
23933	F	Morona Santiago	Vía Gualaquiza-Sig-Sig	-3.18	-78.8	3525	13
26367	F	Azuay	Gualaceo-Plan de Milagro	-3	-78.66	3372	11
26368	F	Azuay	Gualaceo-Plan de Milagro	-3	-78.66	3372	11
26369	F	Azuay	Gualaceo-Plan de Milagro	-3	-78.66	3372	11
26370	F	Azuay	Gualaceo-Plan de Milagro	-3	-78.66	3372	11
26371	F	Azuay	Gualaceo-Plan de Milagro	-3	-78.66	3372	11
26373	F	Azuay	Gualaceo-Plan de Milagro	-3	-78.66	3372	11
26375	F	Azuay	Gualaceo-Plan de Milagro	-3	-78.66	3372	11
45289	F	Azuay	Patacocha, Sigsig	-3.12	-79.07	3650	10
56533	F	Azuay	Laguna Patococha	-3.12	-79.07	3342	10
56534	F	Azuay	Laguna Patococha	-3.12	-79.07	3342	10
56536	F	Azuay	Laguna Patococha	-3.12	-79.07	3342	10
45129	G	El Oro	Chillacocha	-3.51	-79.61	3163	14
45155	G	El Oro	Chillacocha	-3.51	-79.61	3163	14
32785	H	Azuay	Bosque Protector Yanuncahi Irquis, Páramo de Quimsacocha	-3.04	-79.21	3758	9
32786	H	Azuay	Bosque Protector Yanuncahi Irquis, Páramo de Quimsacocha	-3.04	-79.21	3758	9
32787	H	Azuay	Bosque Protector Yanuncahi Irquis, Páramo de Quimsacocha	-3.04	-79.21	3758	9
32788	H	Azuay	Bosque Protector Yanuncahi Irquis, Páramo de Quimsacocha	-3.04	-79.21	3758	9
32791	H	Azuay	Bosque Protector Yanuncahi Irquis, Páramo de Quimsacocha	-3.04	-79.21	3758	9

QCAZ	Clade	Province	Locality	Latitude	Longitude	Altitude	L.M.
32792	H	Azuay	Bosque Protector Yanuncahi Irquis, Páramo de Quimsacocha	-3.04	-79.21	3758	9
43018	H	Azuay	Girón, Parroquia San Gregorio, Páramo de Quimsacocha	-2.91	-79.28	3766	8
43048	H	Azuay	Girón, Parroquia San Gregorio, Páramo de Quimsacocha	-2.91	-79.28	3766	8
43050	H	Azuay	Cuenca, Páramo de Quimsacocha	-2.91	-79.28	3766	8
43054	H	Azuay	Cuenca, Páramo de Quimsacocha	-2.91	-79.28	3766	8
43055	H	Azuay	Cuenca, Páramo de Quimsacocha	-2.91	-79.28	3766	8
43045	H	Azuay	Cuenca, Páramo de Quimsacocha	-2.91	-79.28	3766	8
42993	H	Azuay	Cuenca, Páramo de Quimsacocha	-2.91	-79.28	3766	8
42994	H	Azuay	Cuenca, Páramo de Quimsacocha	-2.91	-79.28	3766	8
43026	H	Azuay	Girón, Parroquia San Gregorio, Páramo de Quimsacocha	-2.91	-79.28	3766	8
43027	H	Azuay	Girón, Parroquia San Gregorio, Páramo de Quimsacocha	-2.91	-79.28	3766	8
43028	H	Azuay	Girón, Parroquia San Gregorio, Páramo de Quimsacocha	-2.91	-79.28	3766	8
43143	H	Azuay	Girón, Parroquia San Gregorio, Páramo de Quimsacocha	-2.91	-79.28	3766	8
46221	I	Napo	Las Carmelas	-0.97	-78.25	2883	3
24860	J	Zamora Chinchipe	Refugio ArcoIris	-3.97	-79.09	2180	17
45554	J	Loja	Parque Nacional Podocarpus, Lagunas el Compadre	-4.17	-79.12	3205	19
45561	J	Loja	Parque Nacional Podocarpus, Lagunas el Compadre	-4.17	-79.12	3205	19
45562	J	Loja	Parque Nacional Podocarpus, Lagunas el Compadre	-4.17	-79.12	3205	19
45563	J	Loja	Parque Nacional Podocarpus, Lagunas el Compadre	-4.17	-79.12	3205	19
45564	J	Loja	Parque Nacional Podocarpus, Lagunas el Compadre	-4.17	-79.12	3205	19
45565	J	Loja	Parque Nacional Podocarpus, Lagunas el Compadre	-4.17	-79.12	3205	19
45567	J	Loja	Parque Nacional Podocarpus, Lagunas el Compadre	-4.17	-79.12	3205	19
45568	J	Loja	Parque Nacional Podocarpus, Lagunas el Compadre	-4.17	-79.12	3205	19
45650	J	Loja	Parque Nacional Podocarpus, Lagunas el Compadre	-4.17	-79.12	3205	19
45652	J	Loja	Parque Nacional Podocarpus, Lagunas el Compadre	-4.17	-79.12	3205	19
45653	J	Zamora Chinchipe	Reserva Tapichalaca, Estación Científica de la reserva	-4.49	-79.12	2567	22

QCAZ	Clade	Province	Locality	Latitude	Longitude	Altitude	L.M.
45663	J	Zamora Chinchipe	Reserva Tapichalaca, Estación Científica de la reserva	-4.49	-79.12	2567	22
46310	J	Zamora Chinchipe	Abra de Zamora	-4	-79.14	-	17
54327	J	Zamora Chinchipe	El Denuncio	-3.84	-79.12	-	16
54330	J	Loja	Shucos	-3.93	-79.22	-	17
54333	J	Zamora Chinchipe	El Denuncio	-3.84	-79.12	-	16
54335	J	Zamora Chinchipe	El Denuncio	-3.84	-79.12	-	16
54336	J	Zamora Chinchipe	El Denuncio	-3.84	-79.12	-	16
54337	J	Zamora Chinchipe	El Denuncio	-3.84	-79.12	-	16
54338	J	Zamora Chinchipe	El Denuncio	-3.84	-79.12	-	16
54339	J	Zamora Chinchipe	El Denuncio	-3.84	-79.12	-	16
32790	K	Azuay	Bosque Protector Yanuncahi Irquis, Páramo de Quimsacocha	-3.04	-79.21	3758	9
45174	L	Azuay	Límite del Parque Nacional Cajas	-2.86	-79.38	2943	7
45175	L	Azuay	Límite del Parque Nacional Cajas	-2.86	-79.38	2943	7
45195	L	Azuay	Límite del Parque Nacional Cajas	-2.86	-79.38	2900	7
46981	L	Azuay	Yumate, Shoupshe	-2.77	-79.43	3504	6
46982	L	Azuay	Yumate, Shoupshe	-2.77	-79.43	3504	6
46984	L	Azuay	Yumate, Shoupshe	-2.77	-79.43	3504	6
46985	L	Azuay	Yumate, Shoupshe	-2.77	-79.43	3504	6
46986	L	Azuay	Yumate, Shoupshe	-2.77	-79.43	3504	6
46987	L	Azuay	Yumate, Shoupshe	-2.77	-79.43	3504	6
46988	L	Azuay	Cochapamba	-2.8	-79.42	3548	6
46989	L	Azuay	Yumate, Shoupshe	-2.77	-79.43	3504	6
46992	L	Azuay	Cochapamba	-2.8	-79.41	3662	6
46993	L	Azuay	Cochapamba	-2.8	-79.41	3662	6
46994	L	Azuay	Cochapamba	-2.8	-79.41	3662	6
46995	L	Azuay	Cochapamba	-2.8	-79.41	3662	6

QCAZ	Clade	Province	Locality	Latitude	Longitude	Altitude	L.M.
46996	L	Azuay	Cochapamba	-2.8	-79.41	3662	6
46999	L	Azuay	Cochapamba	-2.8	-79.42	3548	6
47000	L	Azuay	Cochapamba	-2.8	-79.42	3548	6
47001	L	Azuay	Cochapamba	-2.8	-79.41	3548	6

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